FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

Anti-E1 levels in NON-responders to IFN treatment

FIGURE 5

Anti-E1 levels in RESPONDERS to IFN treatment
SERIES 1
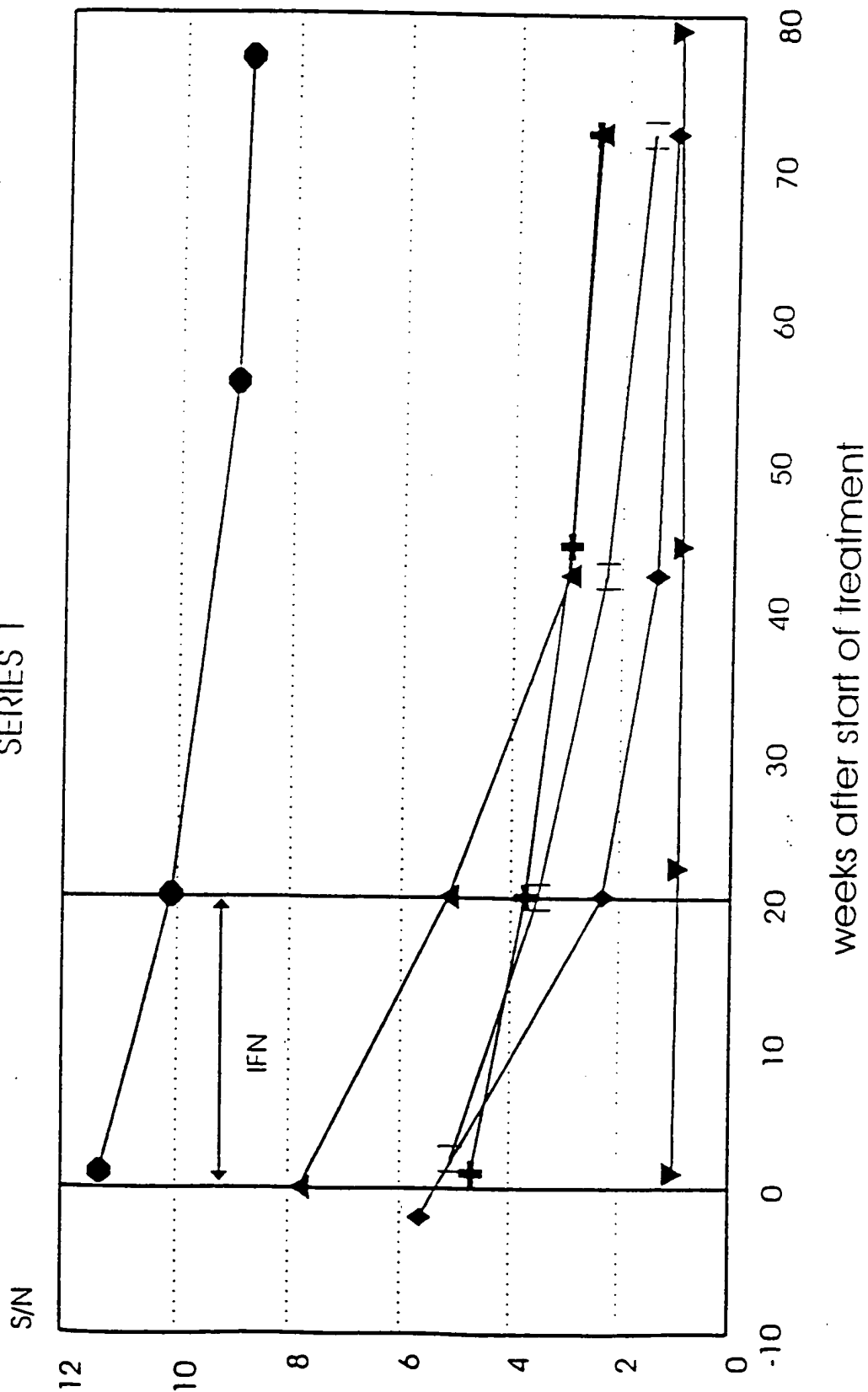
Anti-E1 levels in patients with COMPLETE response to IFN

SERIES 2
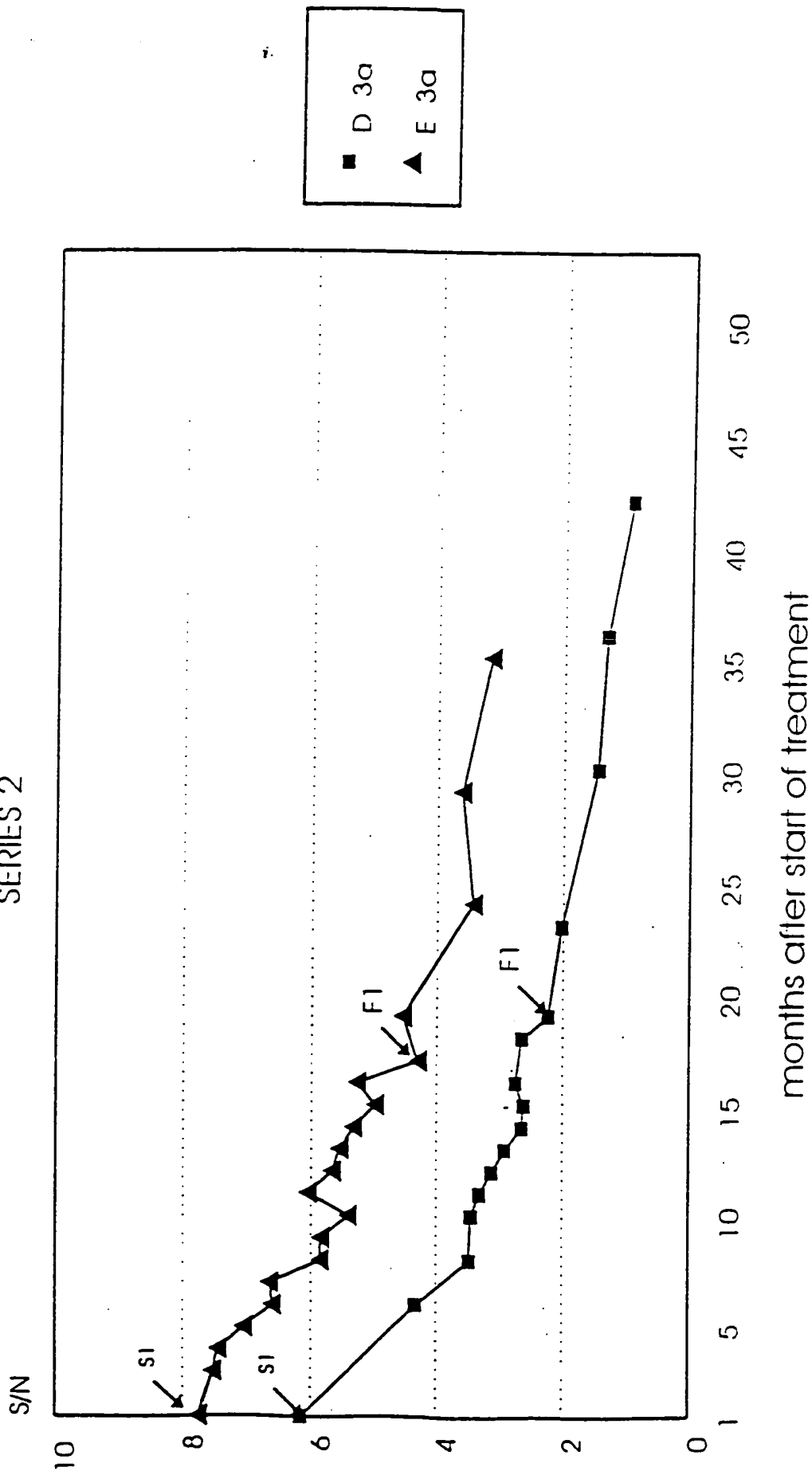
FIGURE 7

Anti-E1 levels in INCOMPLETE responders to IFN treatment
SERIES 2
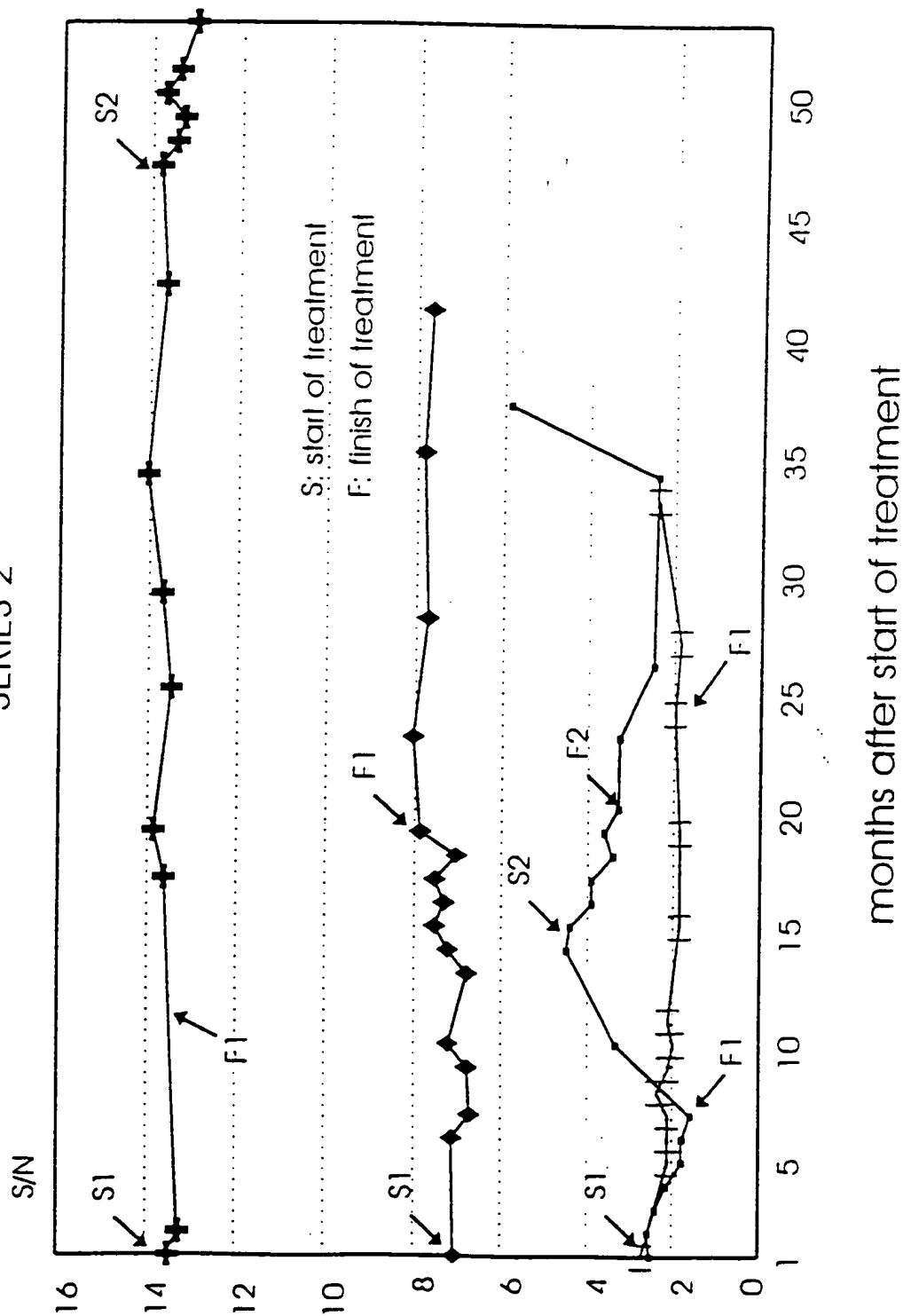
FIGURE 8

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Anti-E2 levels in NON-RESPONDERS to IFN treatment

SERIES 1

▪	G 1b
■	J 1b
■	K 1b
	N 3a
	Q 3a

S/N

IFN

weeks after start of treatment
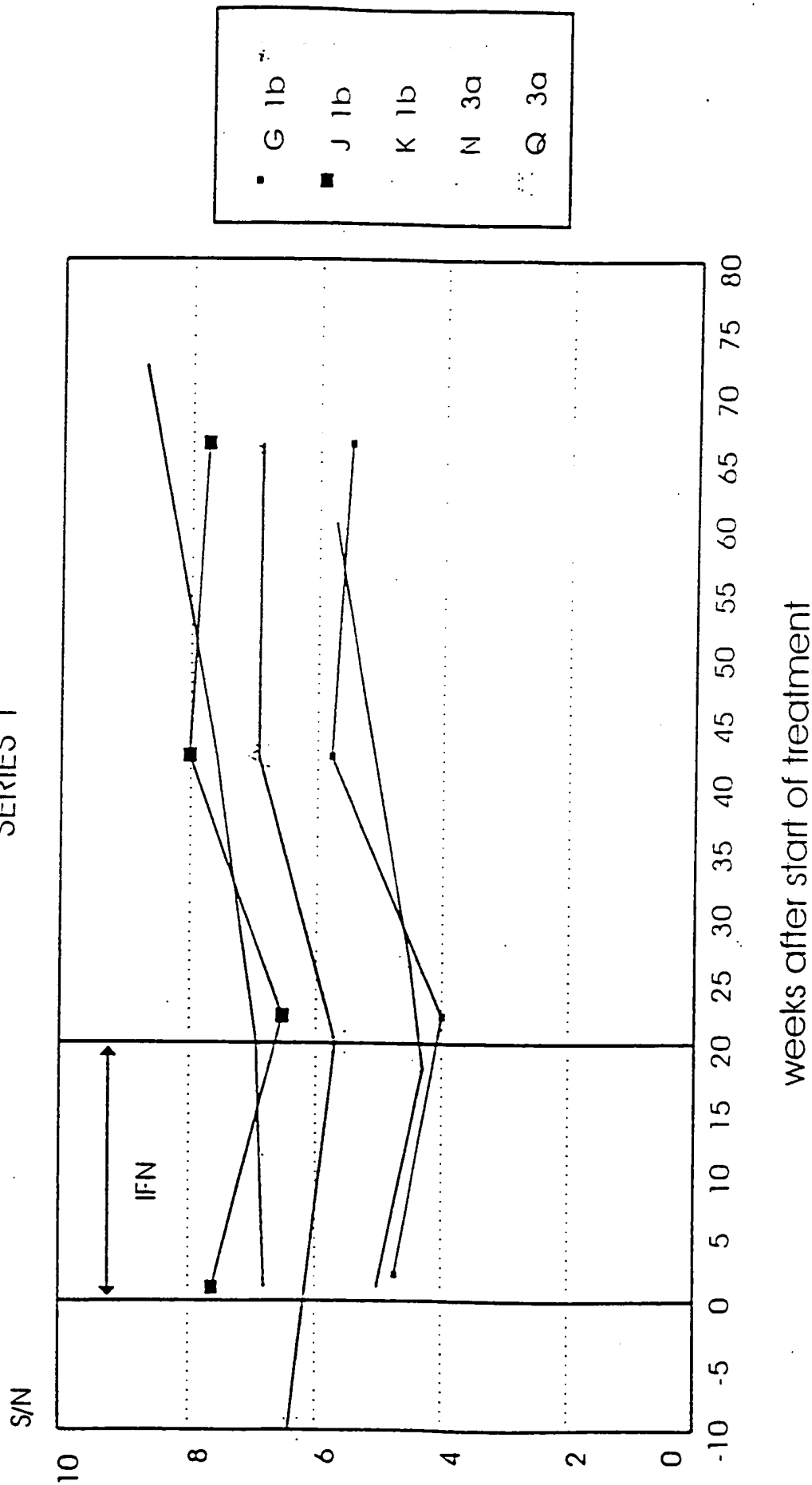
FIGURE 9

Anti-E2 levels in RESPONDERS to IFN treatment

SERIES 1

weeks after start of treatment

Legend:
- H 1b
- + I 1b
- ◆ L 1b
- ◀ M 1b
- ● O 3a
- ▶ P 1b
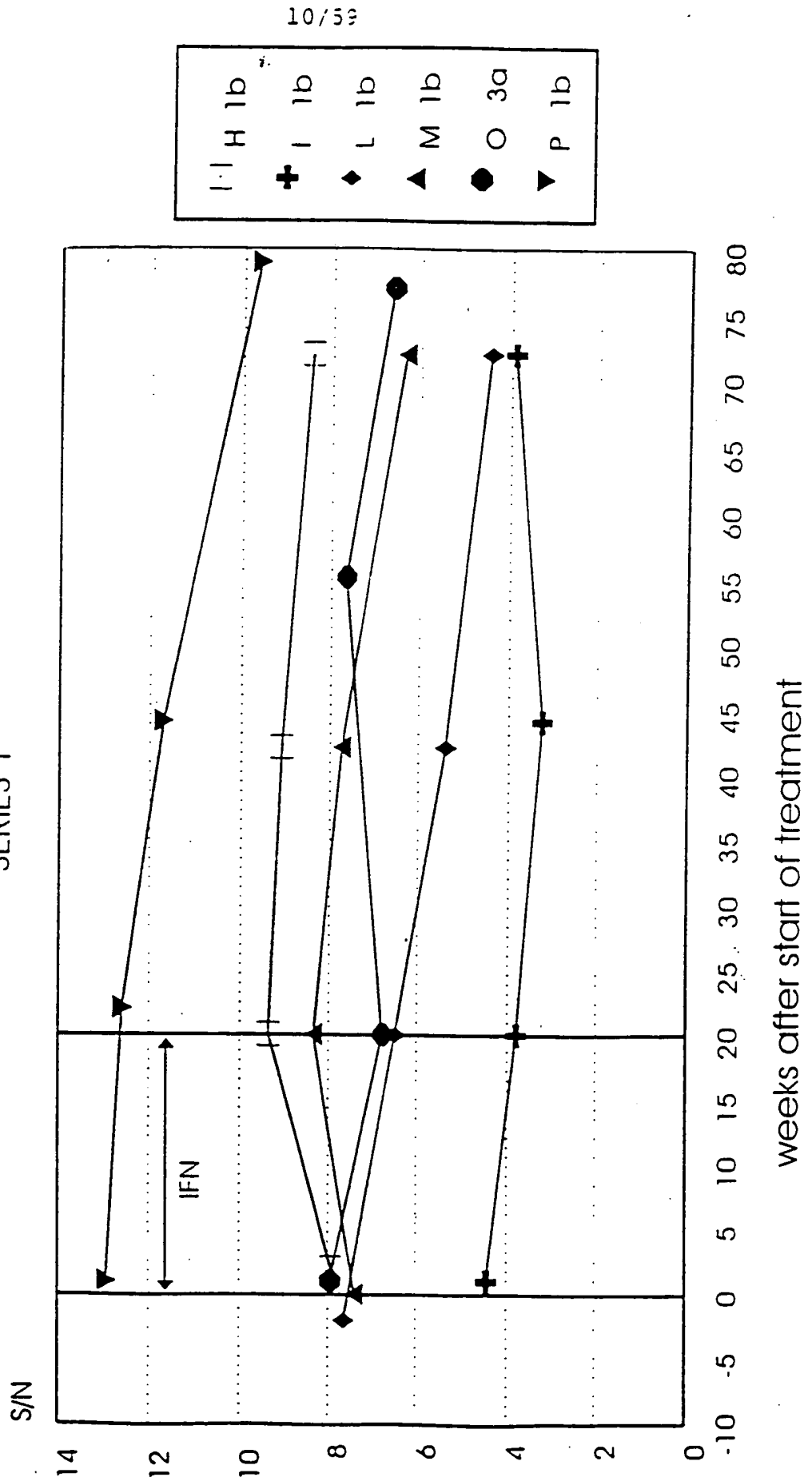
Anti-E2 levels in INCOMPLETE responders to IFN treatment
SERIES 2

FIGURE 11

Legend:
- A 1b NR
- B 1b NR
- C 3a RR
- F 1b PR
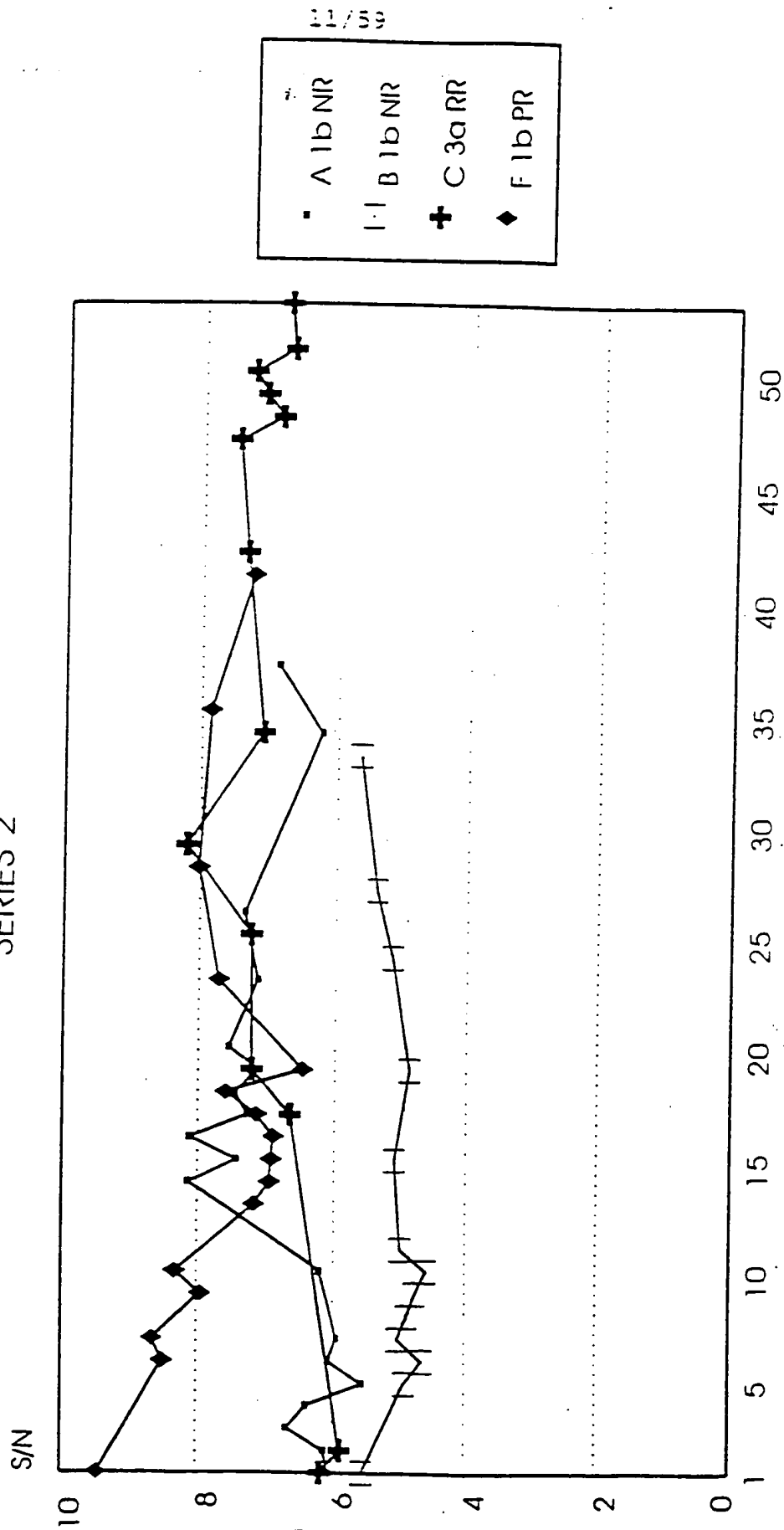
Anti-E2 levels in COMPLETE responders to IFN treatment

SERIES 2

F : finish of treatment

months after start of treatment

FIGURE 12

S/N

■ D 3a CR
▲ E 3a CR
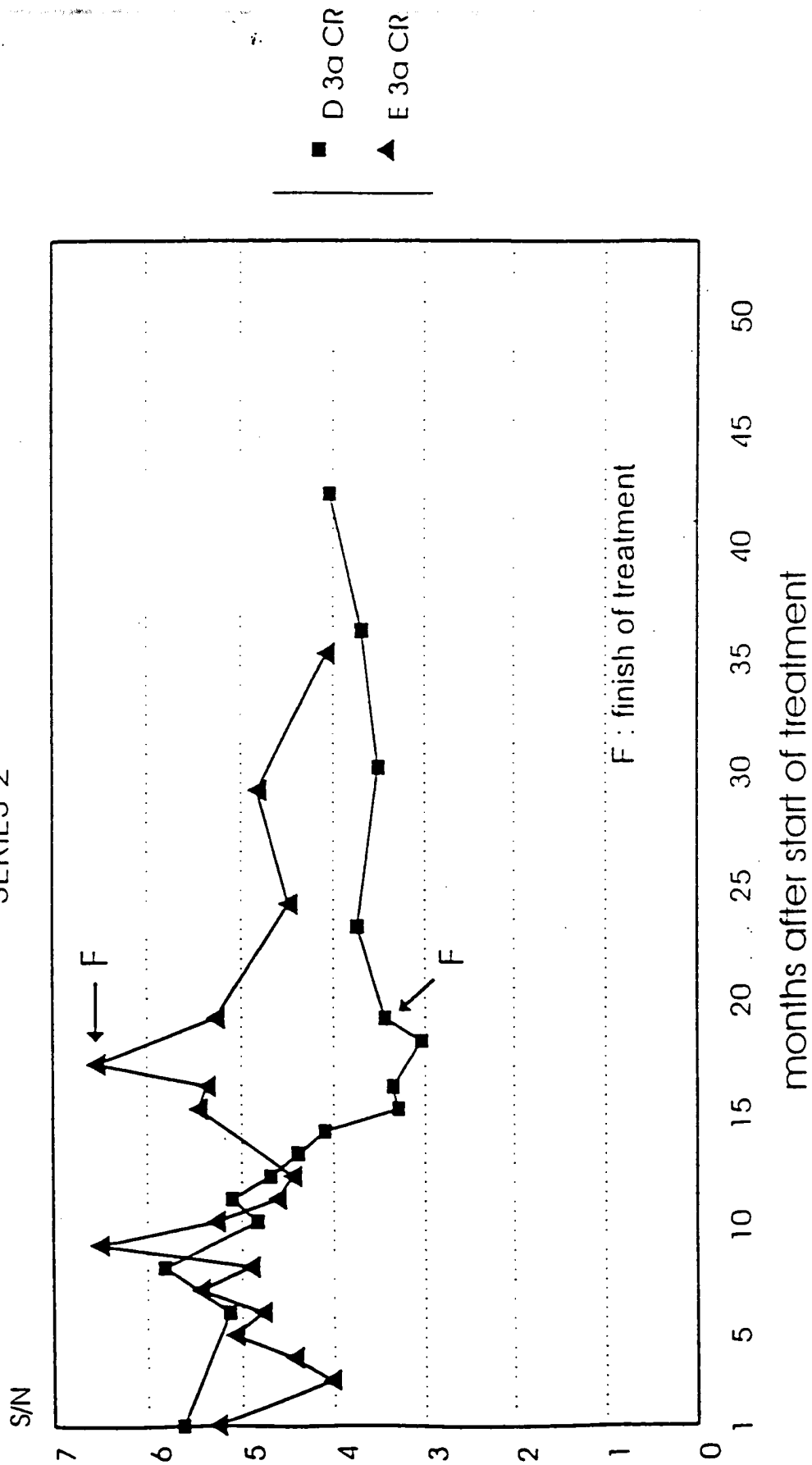
FIGURE 13

Human anti-E1 reactivity competed with peptides
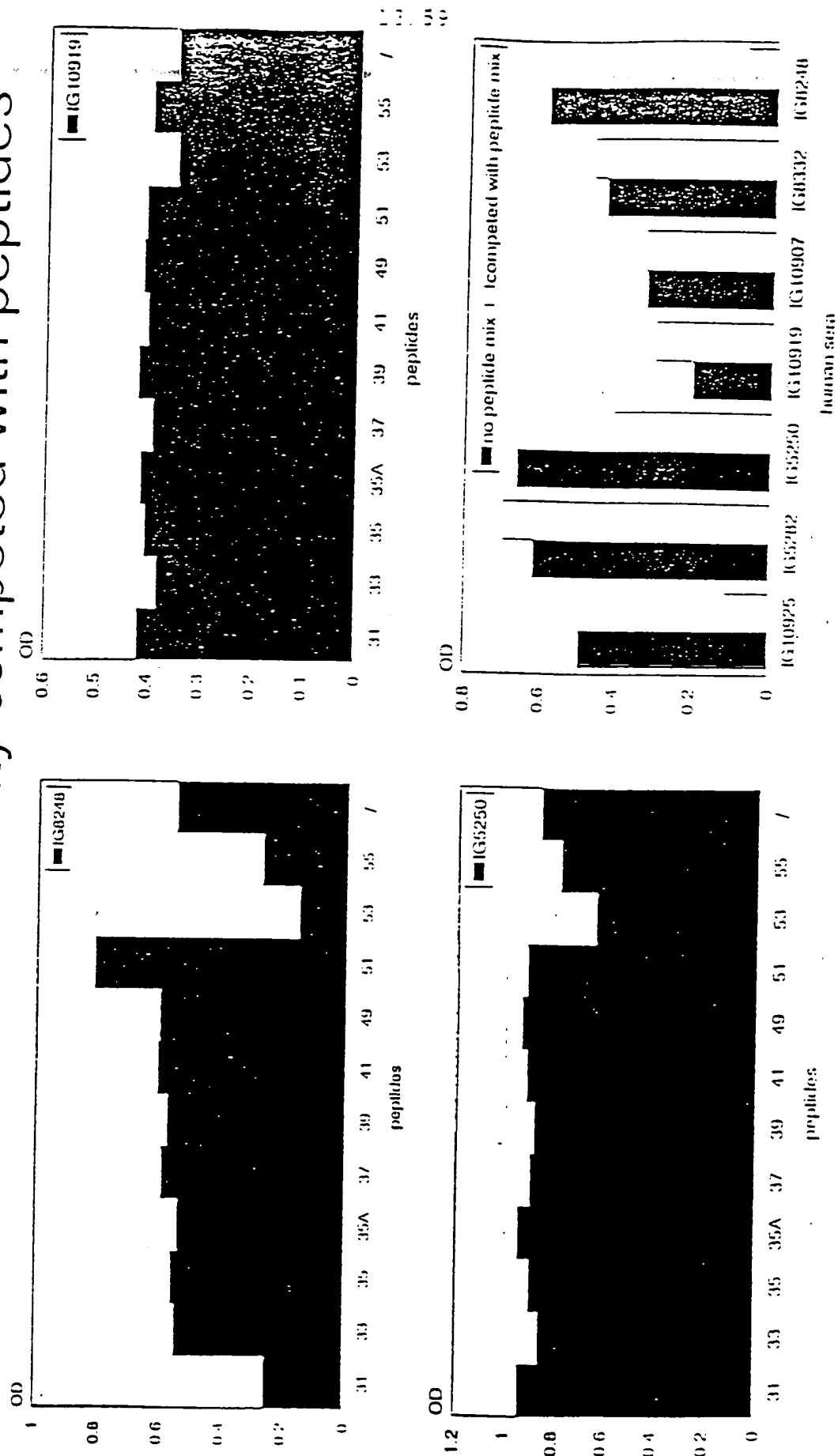
FIGURE 14

Competition of reactivity of anti-E1 Mabs with peptides
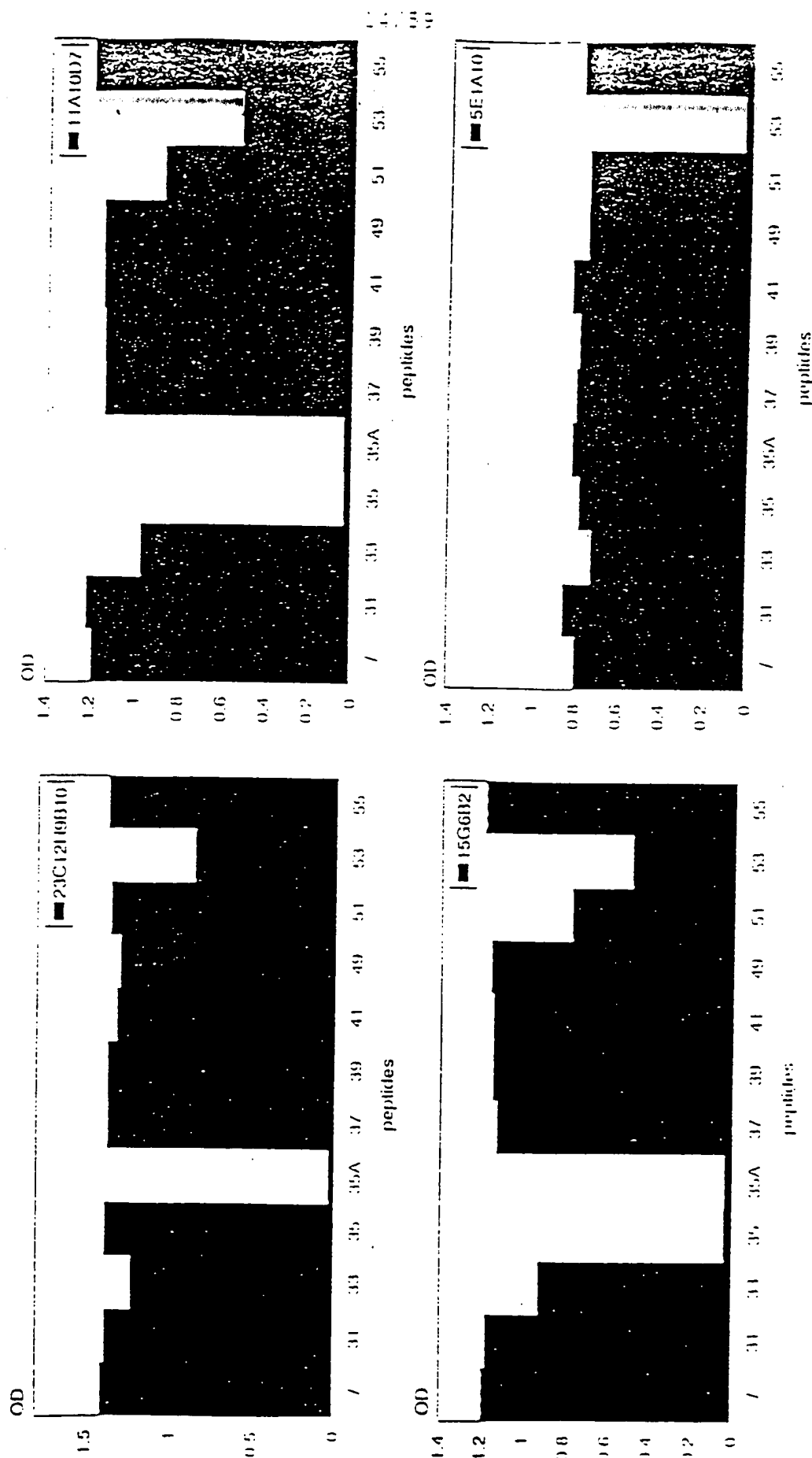
Anti-E1 (epitope 1) levels in NON-RESPONDERS to IFN treatment
SERIES 1
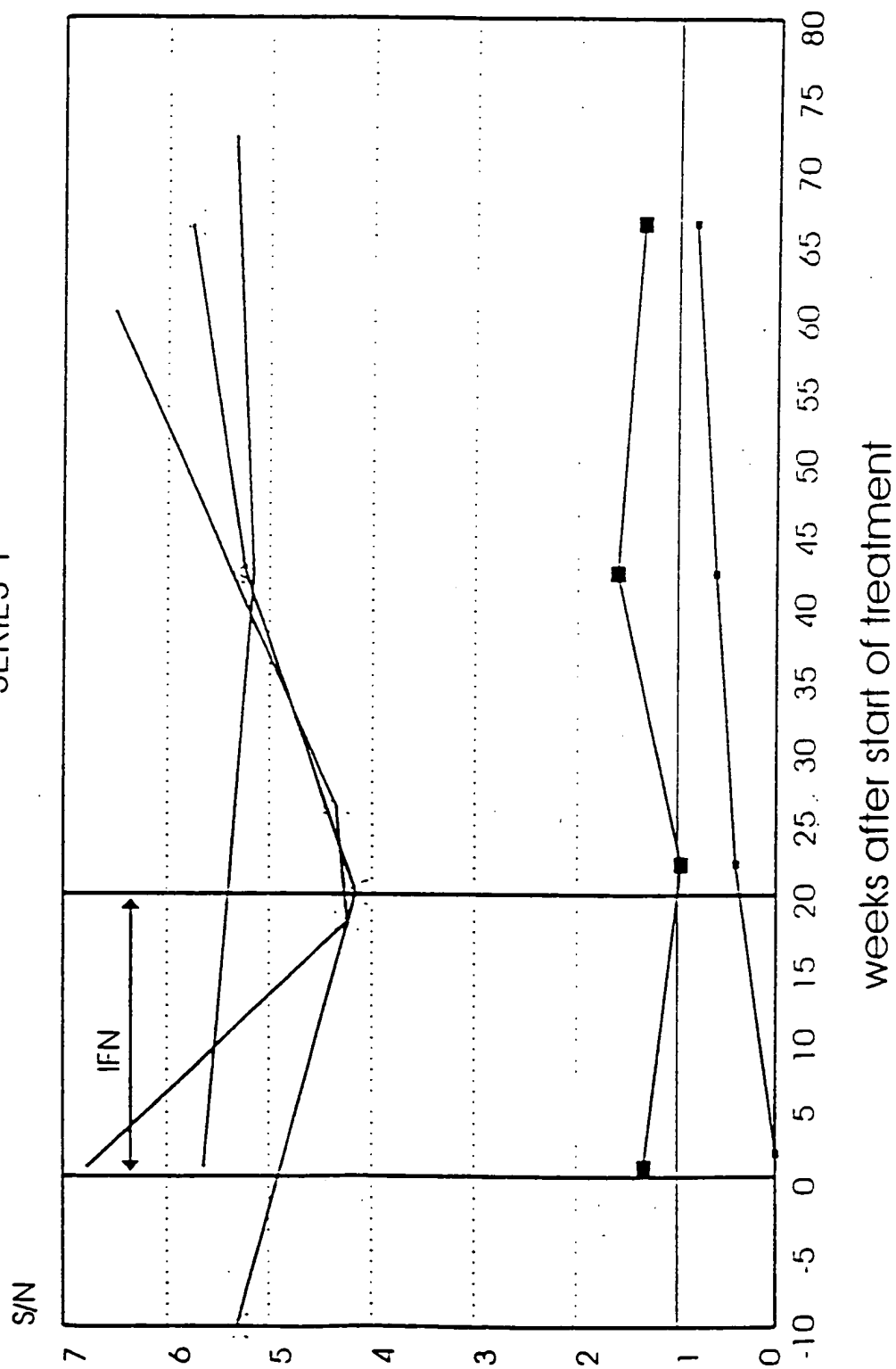
FIGURE 15

Anti-E1 (epitope 1) levels in RESPONDERS to IFN treatment
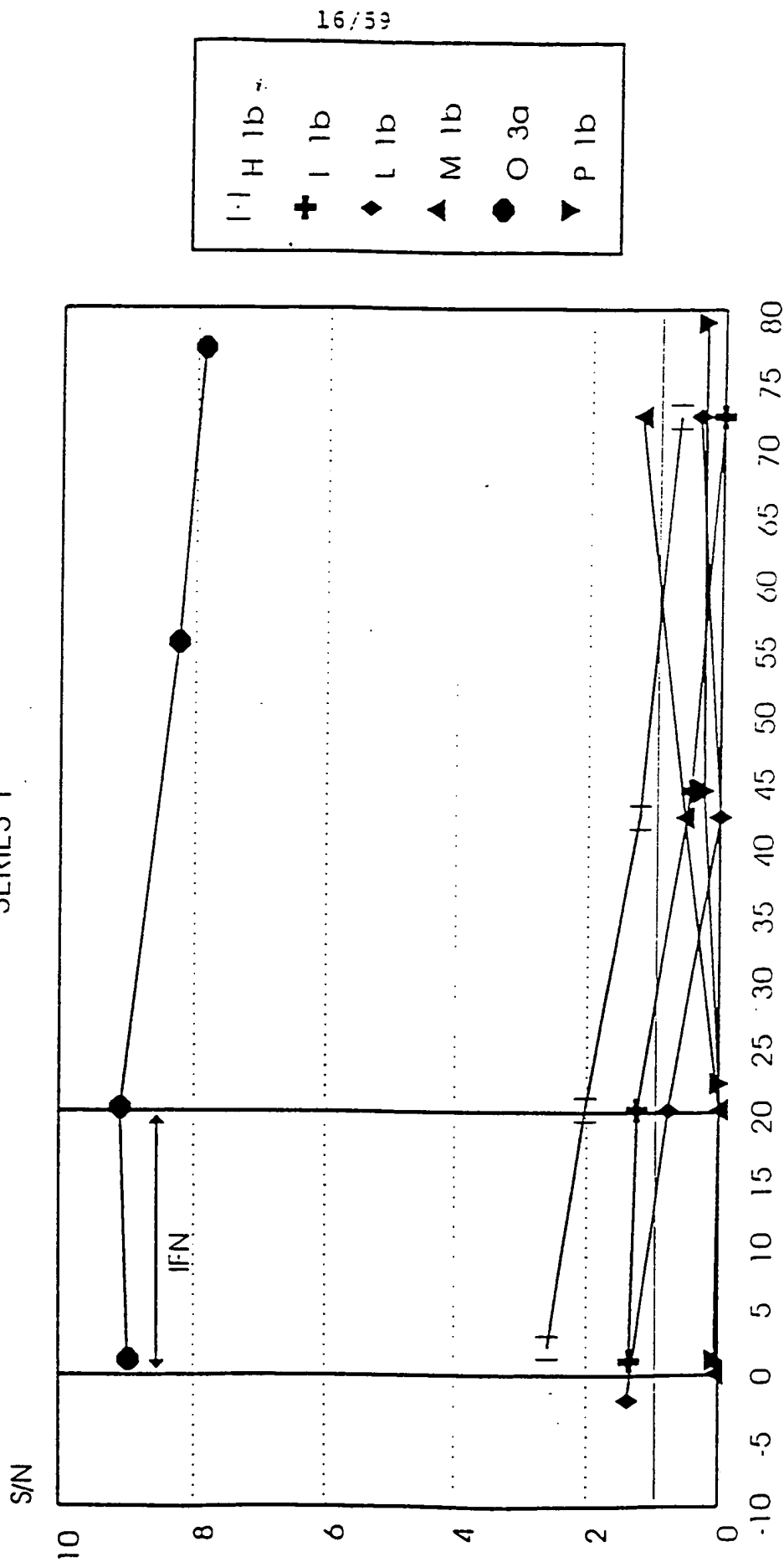
nti-E1 (epitope 2) levels in NON-RESPONDERS to IFN treatment
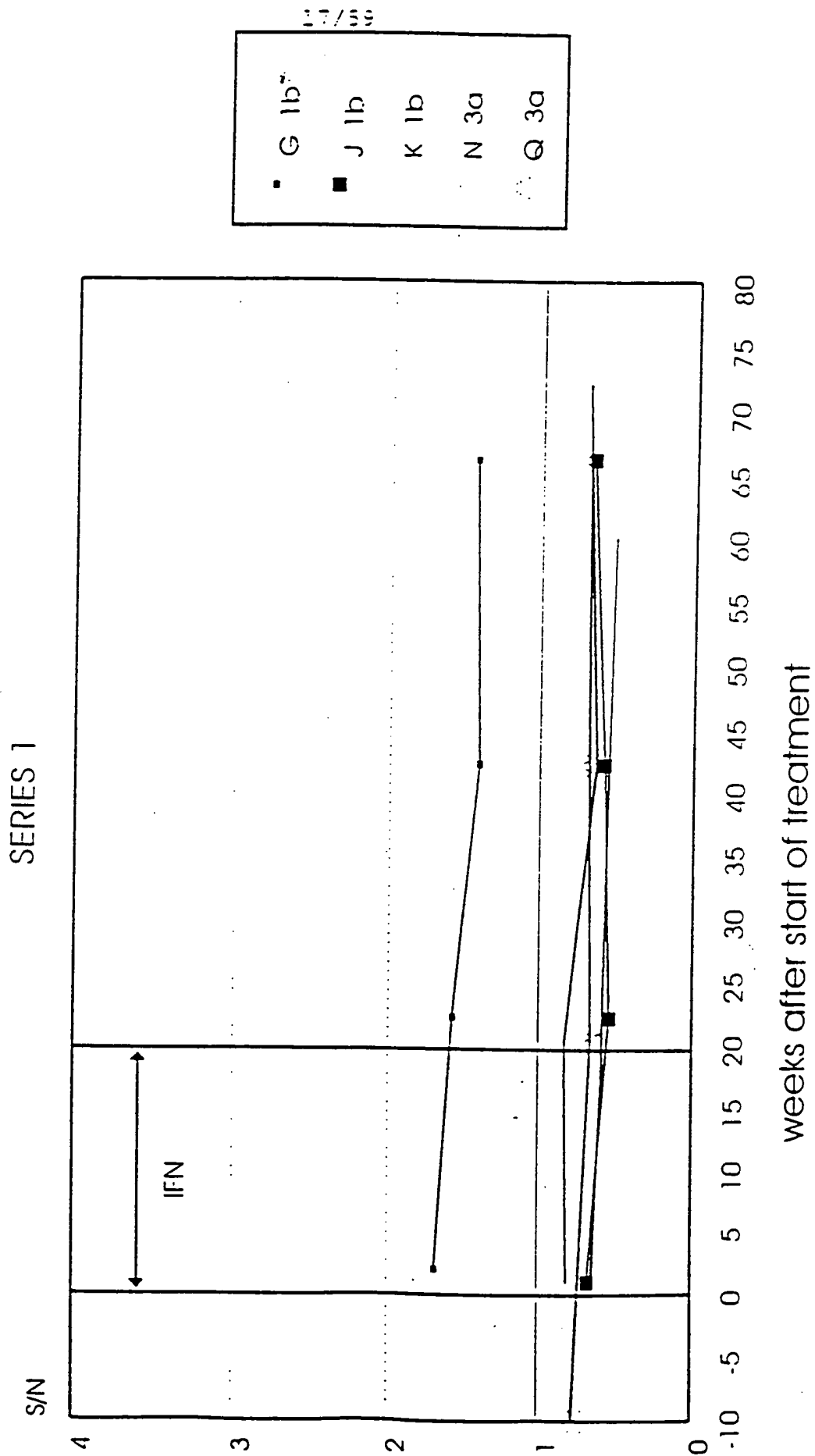
SERIES 1

Anti-E1 (epitope 2) levels in RESPONDERS to IFN treatment

SERIES 1
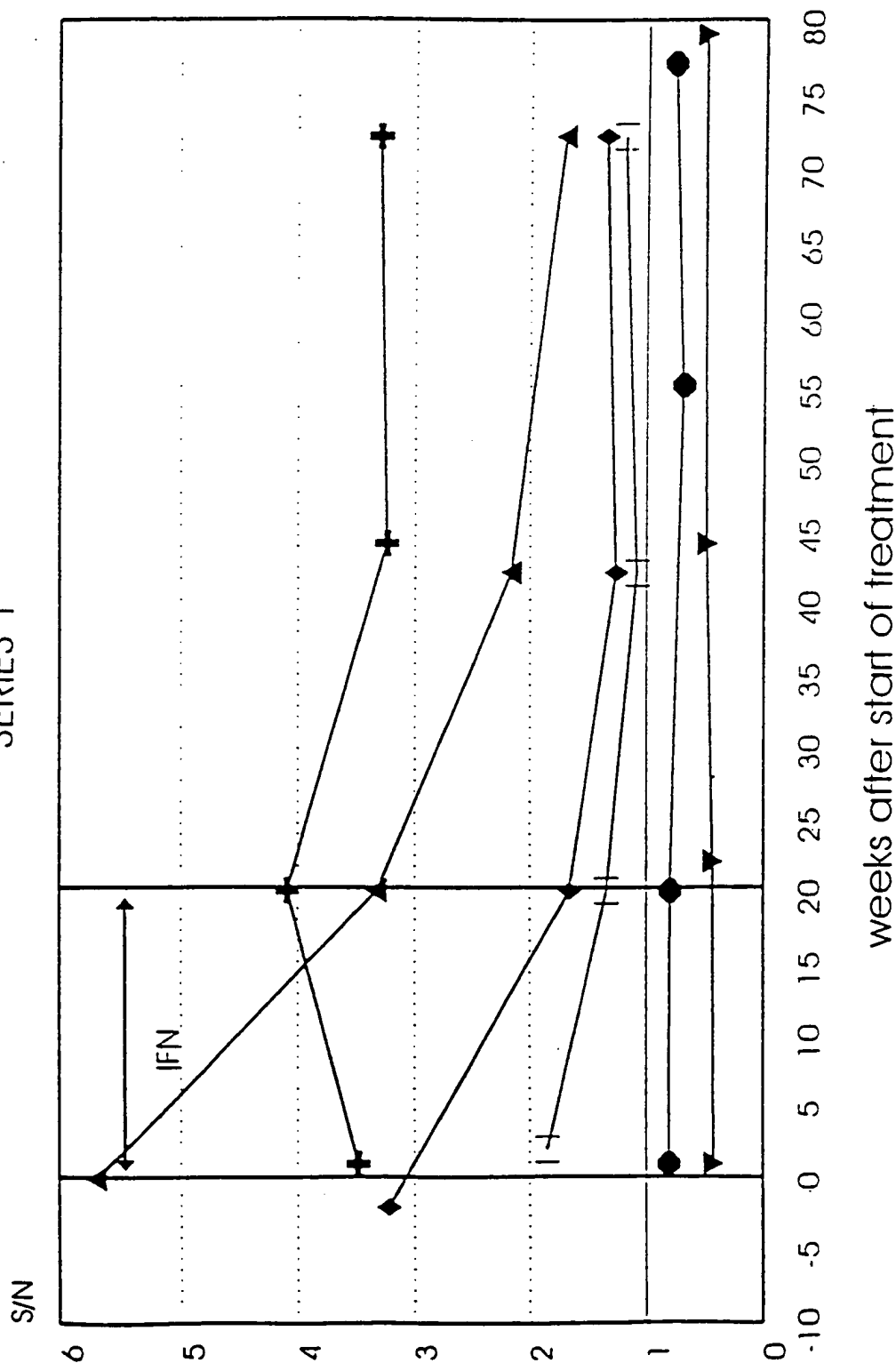
FIGURE 19

Competition of reactivity of anti-E2 Mabs with peptides
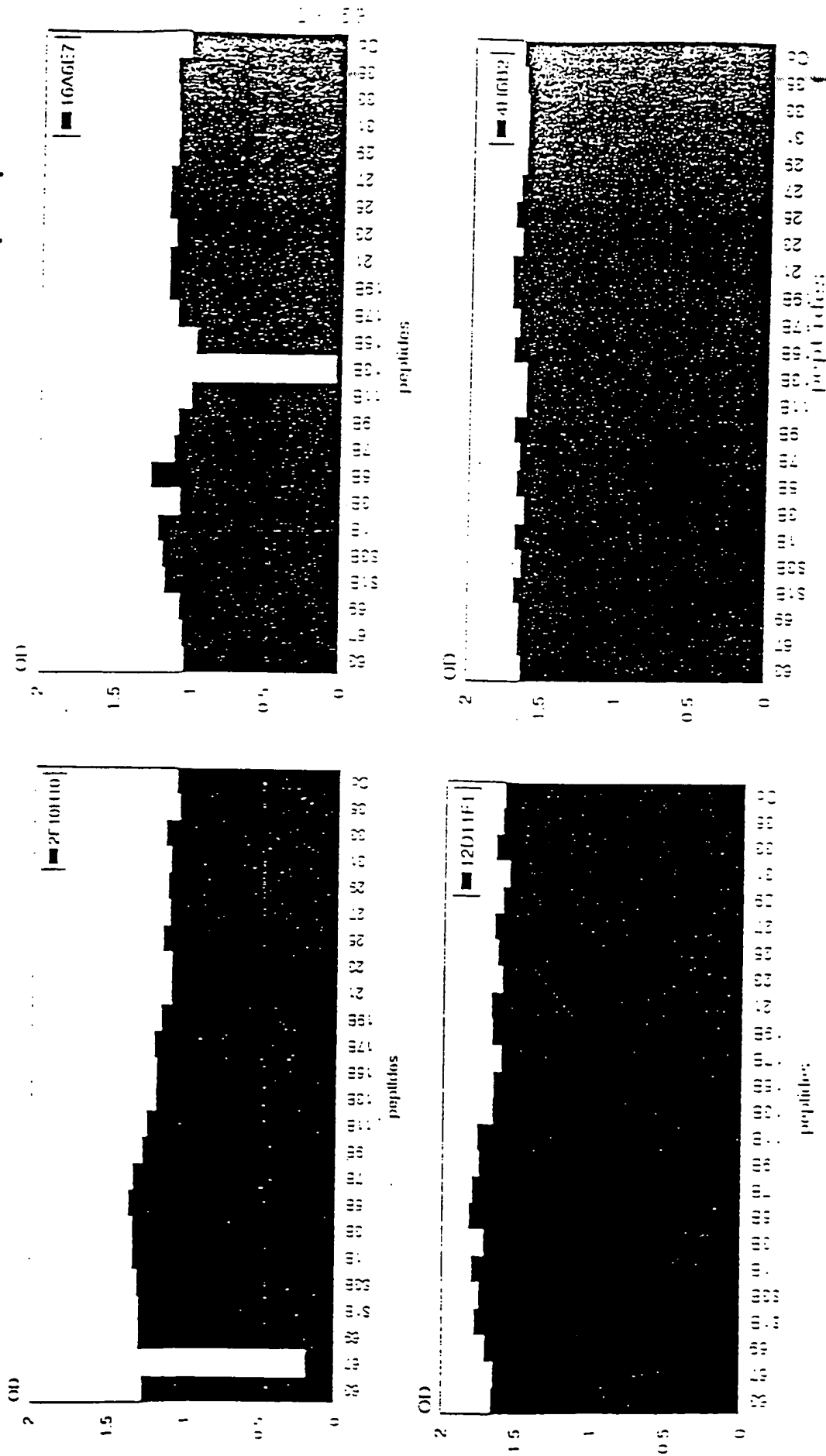
FIGURE 20

Human anti-E2 reactivity competed with peptides
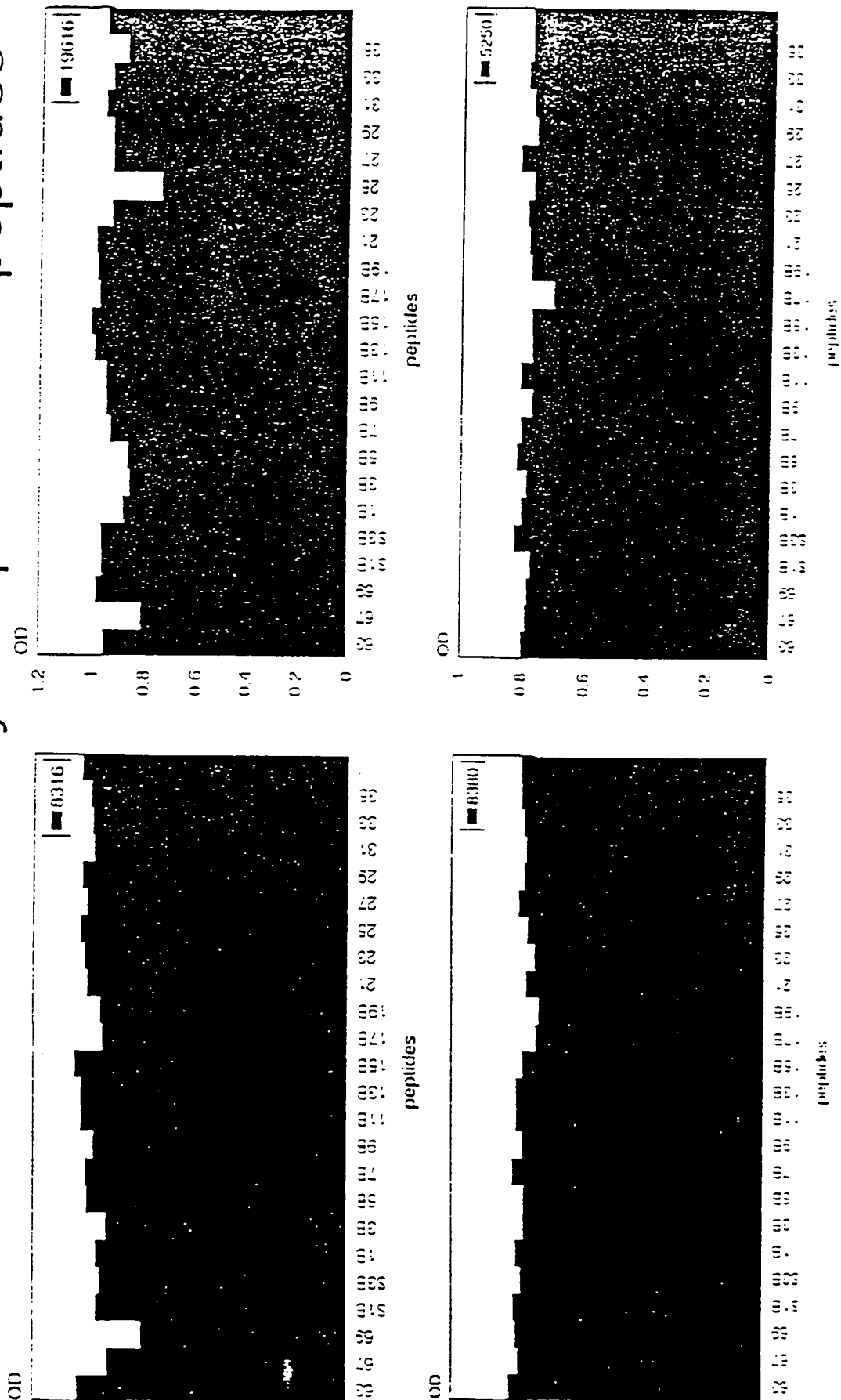
Figure 21

5'   GGCATGCAAGCTTAATTAATT3' (SEQ ID NO 1)

3'ACGTCCGTACGTTCGAATTAATTAATCGA5' (SEQ ID NO 94)

5'CCGGGGAGGCCTGCACGTGATCGAGGGCAGACACCATCACCACCATCACTAATAGT
TAATTAACTGCA 3' (SEQ ID NO 2)

3'CCTCCGGACGTGCACTAGCTCCCGTCTGTGGTAGTGGTGGTAGTGATTATCAATTAATTG
5'   (SEQ ID NO 95)

SEQ ID NO 3 (HCCI9A)

ATGCCCGGTTGCTCTTTCTCTATCTTCCTCTTGGCTTTACTGTCCTGTCTGACCATTCCA
GCTTCCGCTTATGAGGTGCGCAACGTGTCCGGGATGTACCATGTCACGAACGACTGCT
CCAACTCAAGCATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT
GCCCTGCGTTCGGGAGAACAACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTC
GCAGCTAGGAACGCCAGCGTCCCCACCACGACAATACGACGCCACGTCGATTTGCTCG
TTGGGGCGGCTGCTCTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTC
CTCGTCTCCCAGCTGTTCACCATCTCGCCTCGCCGGCATGAGACGGTGCAGGACTGCA
ATTGCTCAATCTATCCCGGCCACATAACAGGTCACCGTATGGCTTGGGATATGATGAT
GAACTGGTCGCCTACAACGGCCCTGGTGGTATCGCAGCTGCTCCGGATCCCACAAGCT
GTCGTGGACATGGTGGCGGGGGCCCATTGGGGAGTCCTGGCGGGCCTCGCCTACTATT
CCATGGTGGGGAACTGGGCTAAGGTTTTGATTGTGATGCTACTCTTTGCTCTCTAATAG

SEQ ID NO 5 (HCCI10A)

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TAAGGTTTTGATTGTGATGCTACTCTTTGCTCCCTAATAG

SEQ ID NO 7 (HCCI11A)

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TCTTCCTCTTGGCTTTACTGTCCTGTCTGACCATTCCAGCTTCCGCTTATGAGGTGCGC
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CCCCACTACGACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTGTT
CCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTCCTCGTCTCCCAGCTGTTCACC
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ACATAACAGGTCACCGTATGGCTTGGGATATGATGATGAACTGGTAATAG

SEQ ID NO 9 (HCCI12A)

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GCCCTGCGTTCGGGAGGGCAACTCCTCCCGTTGCTGGGTGGCGCTCACTCCCACGCTC
GCGGCCAGGAACGCCAGCGTCCCCACAACGACAATACGACGCCACGTCGATTTGCTC
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CCTTGTTTCCCAGCTGTTCACCTTCTCACCTCGCCGGCATCAAACAGTACAGGACTGCA
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GAACTGGTCCTAATAG

SEQ ID NO 11 (HCCI13A)

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CCTTGTTTCCCAGCTGTTCACCTTCTCACCTCGCCGGCATCAAACAGTACAGGACTGCA
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GAACTGGTAATAG

SEQ ID NO 13 (HCCl17A)
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TCTTCCTCTTGGCTTTACTGTCCTGTCTAACCATTCCAGCTTCCGCTTACGAGGTGCGC
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CCCCACTACAACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTGTT
CCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTCCTCGTCTCCCAGCTGTTCACC
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ACATAACGGGTCACCGTATGGCTTGGGATATGATGATGAACTGGTACTAATAG

SEQ ID NO 15 (HCPr51)
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SEQ ID NO 16 (HCPr52)
ATGTTGGGTAAGGTCATCGATACCCT

SEQ ID NO 17 (HCPr53)
CTATTAGGACCAGTTCATCATCATATCCCA

SEQ ID NO 18 (HCPr54)
CTATTACCAGTTCATCATCATATCCCA

SEQ ID NO 19 (HCPr107)
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SEQ ID NO 20 (HCPr108)

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SEQ ID NO 21 (HCCI37)

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ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
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CACCGTATGGCTTGGGATATGATGATGAACTGGTCGCCTACAACGGCCCTGGTGGTAT
CGCAGCTGCTCCGGATCCCACAAGCTGTCGTGGACATGGTGGCGGGGGCCCATTGGGG
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SEQ ID NO 23 (HCCI38)

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TAG

SEQ ID NO 25 (HCCI39)

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SEQ ID NO 27 (HCCI40)

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SEQ ID NO 31 (HCCl63)

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SEQ ID NO 33 (HCPr109)

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SEQ ID NO 34 (HCPr72)

CTATTATGGTGGTAAKGCCARCARGAGCAGGAG

SEQ ID NO 35 (HCCL22A)

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AGGGTTCTTTGCCGCACTATTCTACAAACACAAATTCAACTCGTCTGGATGCCCAGAG

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SEQ ID NO 37 (HCCI41)

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SEQ ID NO 39 (HCCI42)

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SEQ ID NO 41 (HCCl43)

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SEQ ID NO 43 (HCCI44)

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SEQ ID NO 45 (HCCL64)

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SEQ ID NO 47 (HCCI65)

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SEQ ID NO 49 (HCCI66)

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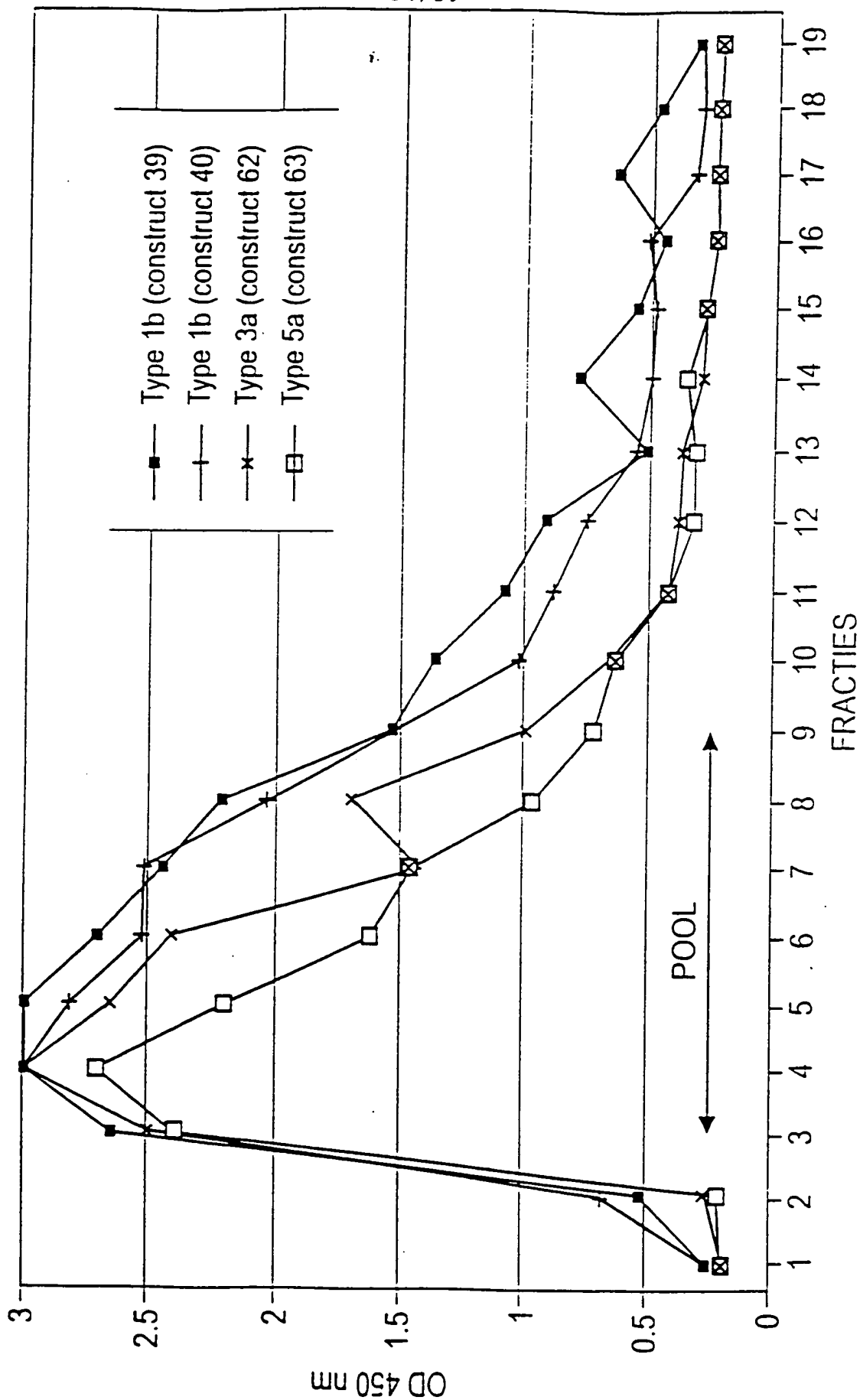
Figure 22

OD measured at 450 nm
construct

Fraction	volume	dilution	39 Type Ib	40 Type Ib	62 Type 5a	63 Type 5a
START	23 ml	1/20	2.517	1.954	1.426	1.142
FLOW THROUGH	23 ml	1/20	0.087	0.085	0.176	0.120
1	0.4 ml	1/200	0.102	0.051	0.048	0.050
2			0.396	0.550	0.090	0.067
3			2.627	2.603	2.481	2.373
4			3	2.967	3	2.694
5			3	2.810	2.640	2.154
6			2.694	2.499	1.559	1.561
7			2.403	2.481	0.347	1.390
8			2.176	1.970	1.624	0.865
9			1.461	1.422	0.887	0.604
10			1.256	0.926	0.543	0.519
11			0.981	0.781	0.294	0.294
12			0.812	0.650	0.249	0.199
13			0.573	0.432	0.239	0.209
14			0.653	0.371	0.145	0.184
15			0.441	0.348	0.151	0.151
16			0.321	0.374	0.098	0.106
17			0.525	0.186	0.099	0.108
18			0.351	0.171	0.083	0.090
19			0.192	0.164	0.084	0.087

34/59

| Fraction | volume | dilution | OD measured at 450 nm construct | | | |
			39 Type 1b	40 Type 1b	62 Type 3a	63 Type 5a
20	250 μl	1/200	0.072	0.130	0.096	0.051
21			0.109	0.293	0.084	0.052
22			0.279	0.249	0.172	0.052
23			0.093	0.151	0.297	0.054
24			0.080	0.266	0.438	0.056
25			0.251	0.100	0.457	0.048
26			3	1.649	0.722	0.066
27			3	3	2.526	0.689
28			3	3	3	2.345
29			3	3	2.849	2.580
30			2.227	1.921	1.424	1.333
31			0.263	0.415	0.356	0.162
32			0.071	0.172	0.154	0.064
33			0.103	0.054	0.096	0.057
34			0.045	0.045	0.044	0.051
35			0.043	0.047	0.045	0.046
36			0.045	0.045	0.049	0.040
37			0.045	0.047	0.046	0.048
38			0.046	0.048	0.047	0.057
39			0.045	0.048	0.050	0.057
40			0.046	0.049	0.048	0.049

FIGURE 25

Figure 2S

Figure 27

Figure 28

Lane 1: Crude Lysate
Lane 2: Flow through Lentil Chromatography
Lane 3: Wash with EMPIGEN Lentil Chromatography
Lane 4: Eluate Lentil Chromatography
Lane 5: Flow through during concentration lentil eluate
Lane 6: Pool of E1 after Size Exclusion Chromatography

Figure 29: Western Blot Analysis with anti-E1 mouse monoclonal 5E1A10
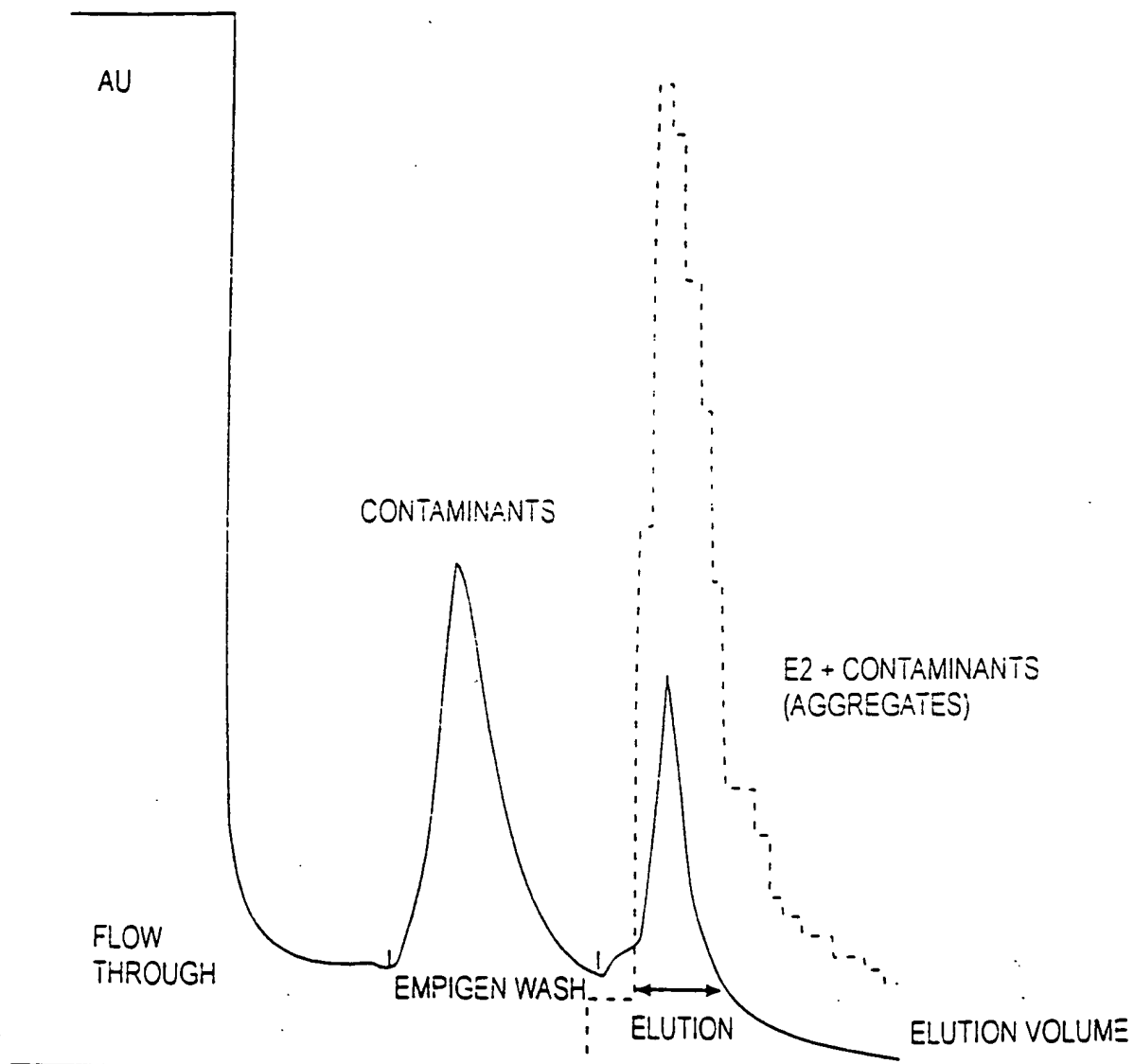
FIGURE 30

A: NON - REDUCED
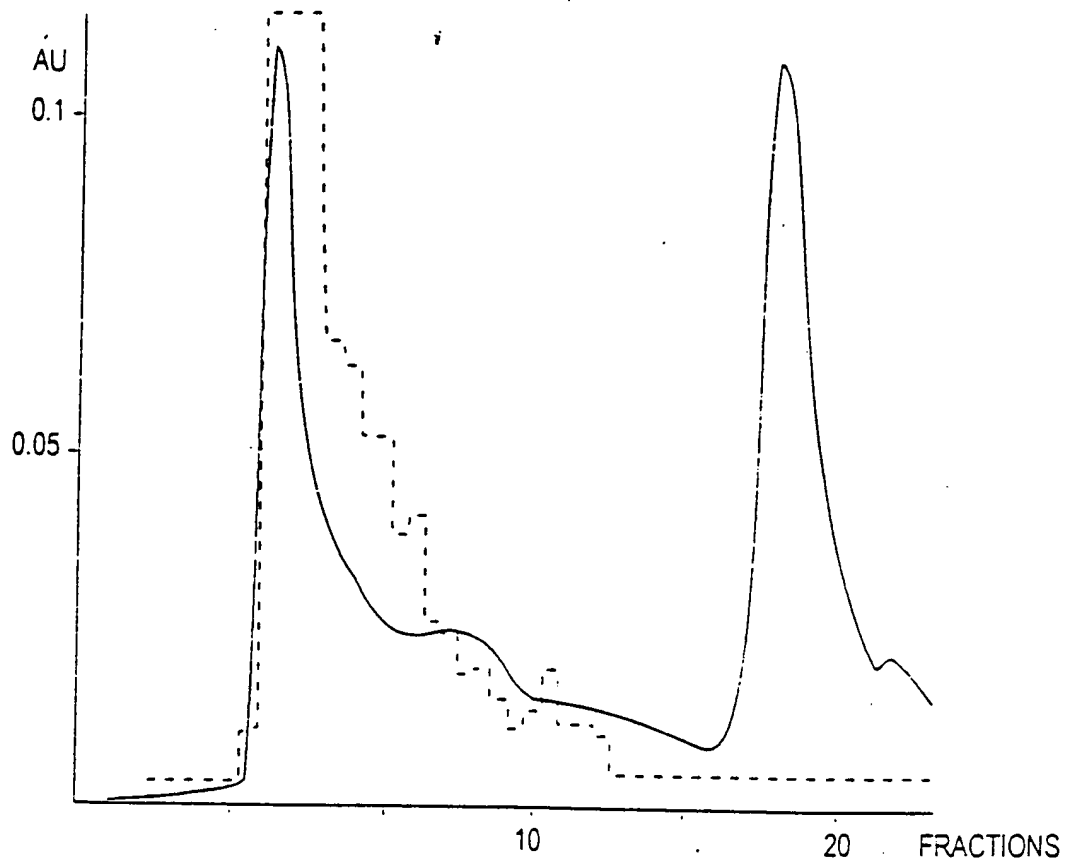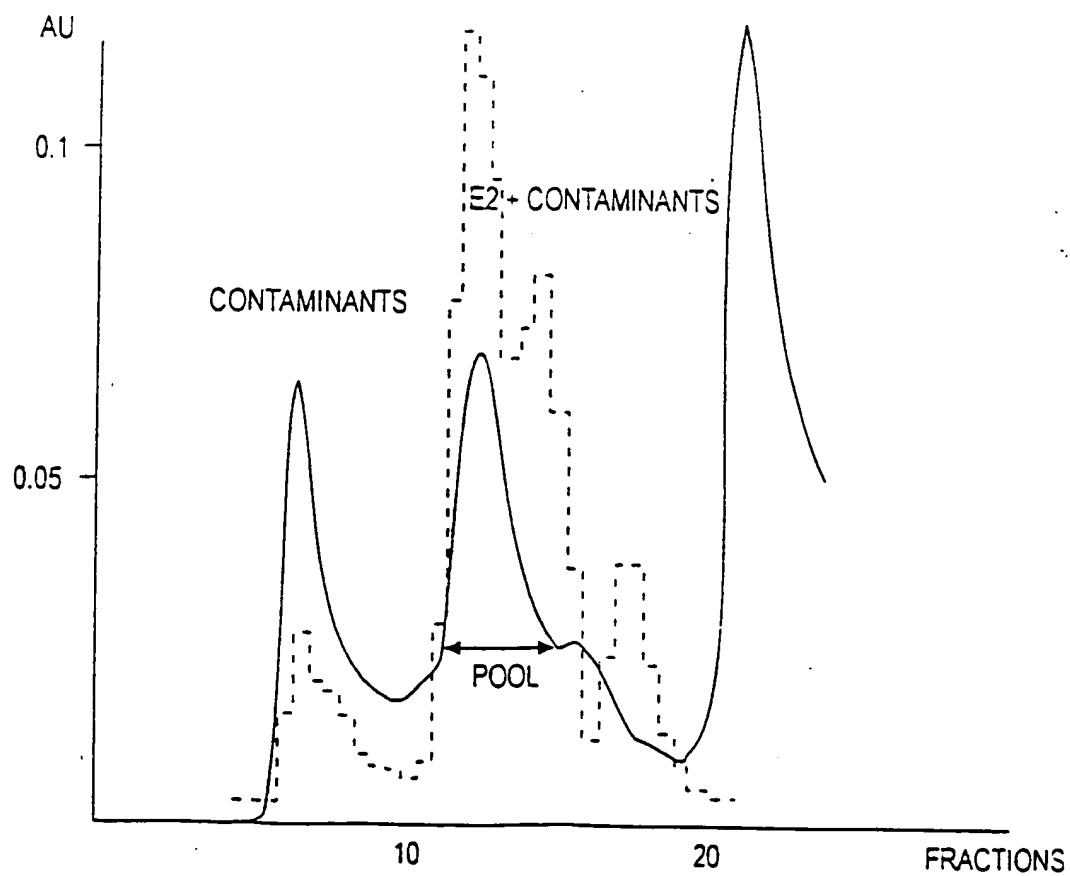
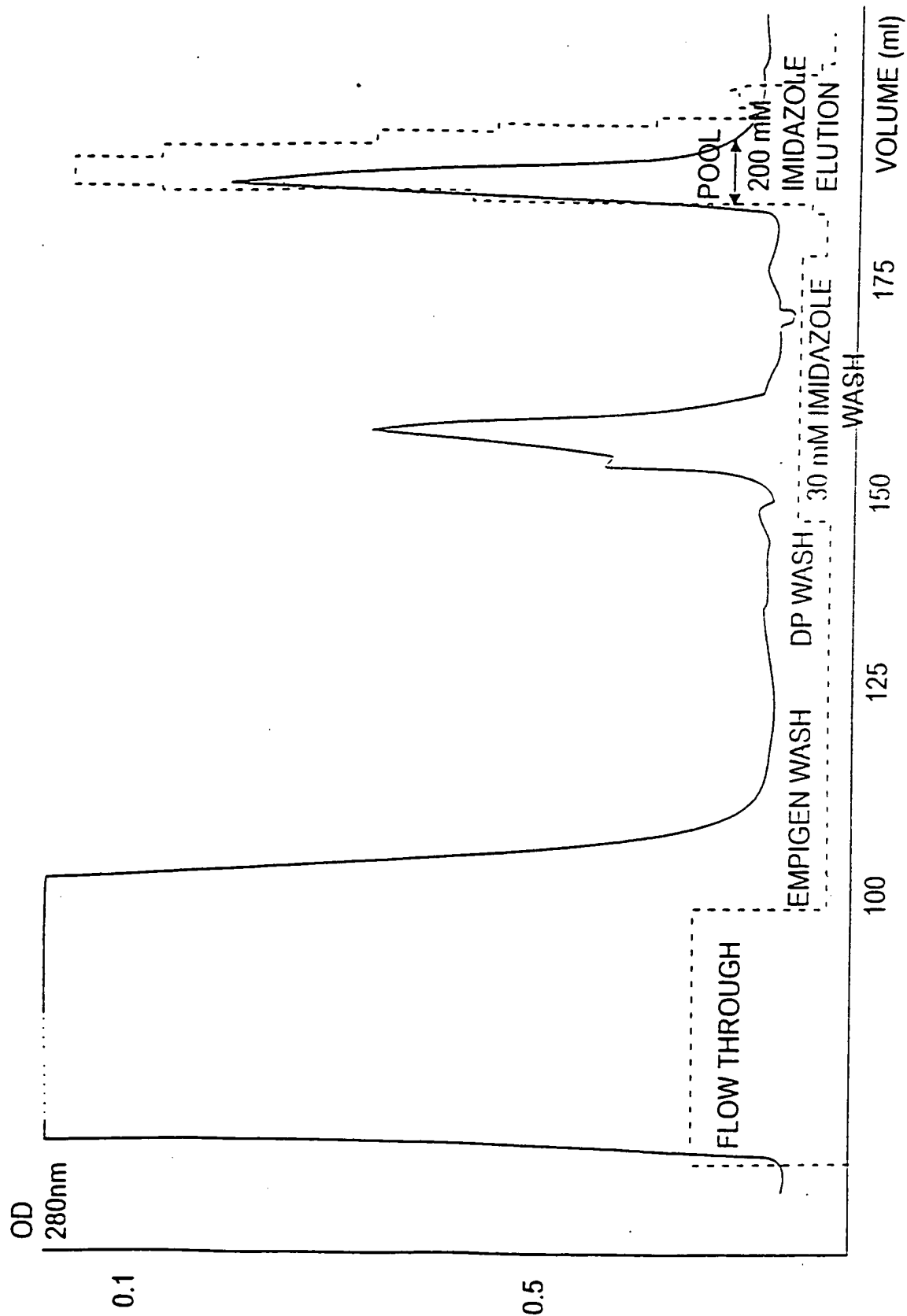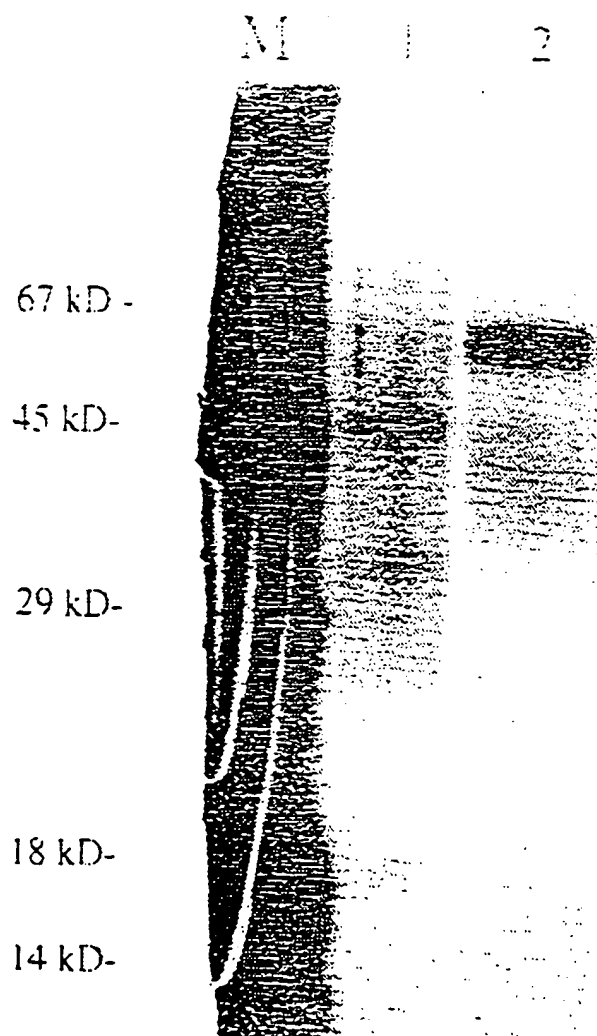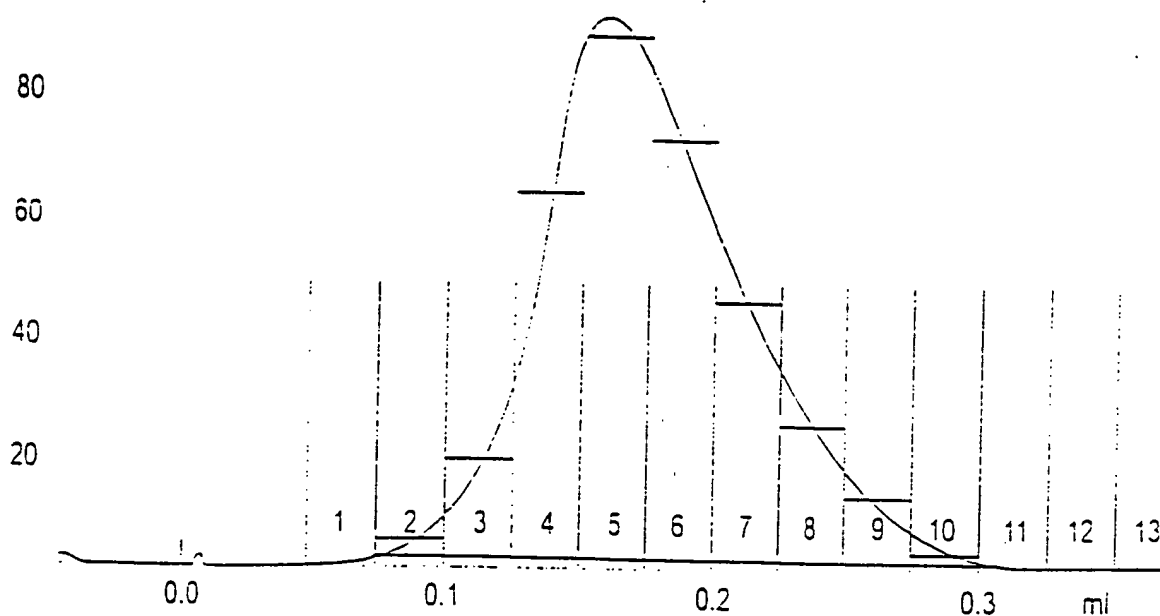E2 + CONTAMINANTS (AGGREGATES)

B : REDUCED

FIGURE 31

FIGURE 32

FIGURE 33:
SILVER STAIN OF PURIFIED E2

M   1   2

67 kD -

45 kD-

29 kD-

18 kD-

14 kD-

1. 30 mM IMIDAZOLE WASH Ni-IMAC
2. 0.5 ug E2

45 59   Figure 34

No.	Ret. (ml)	Peak start (ml)	Peak end (ml)	Dur (ml)	Area (ml*mAU)	Height (mAU)
1	-0.45	-0.46	-0.43	0.04	0.0976	4.579
2	1.55	0.75	3.26	2.51	796.4167	889.377
3	3.27	3.26	3.31	0.05	0.0067	0.224
4	3.33	3.32	3.33	0.02	0.0002	0.018

Total number of detected peaks = 4
Total Area above baseline = 0.796522 ml*AU
Total area in evaluated peaks = 0.796521 ml*AU
Ratio peak area / total area = 0.999999
Total peak duration = 2.613583 ml

FIGURE 35A

Core 1 Ab NR

Core 1 Ab LTR

Core 2 Ab NR

Core 2 Ab LTR

E2HVR Ab NR

E2HVR Ab LTR

NS3 Ab NR

NS3 Ab LTR
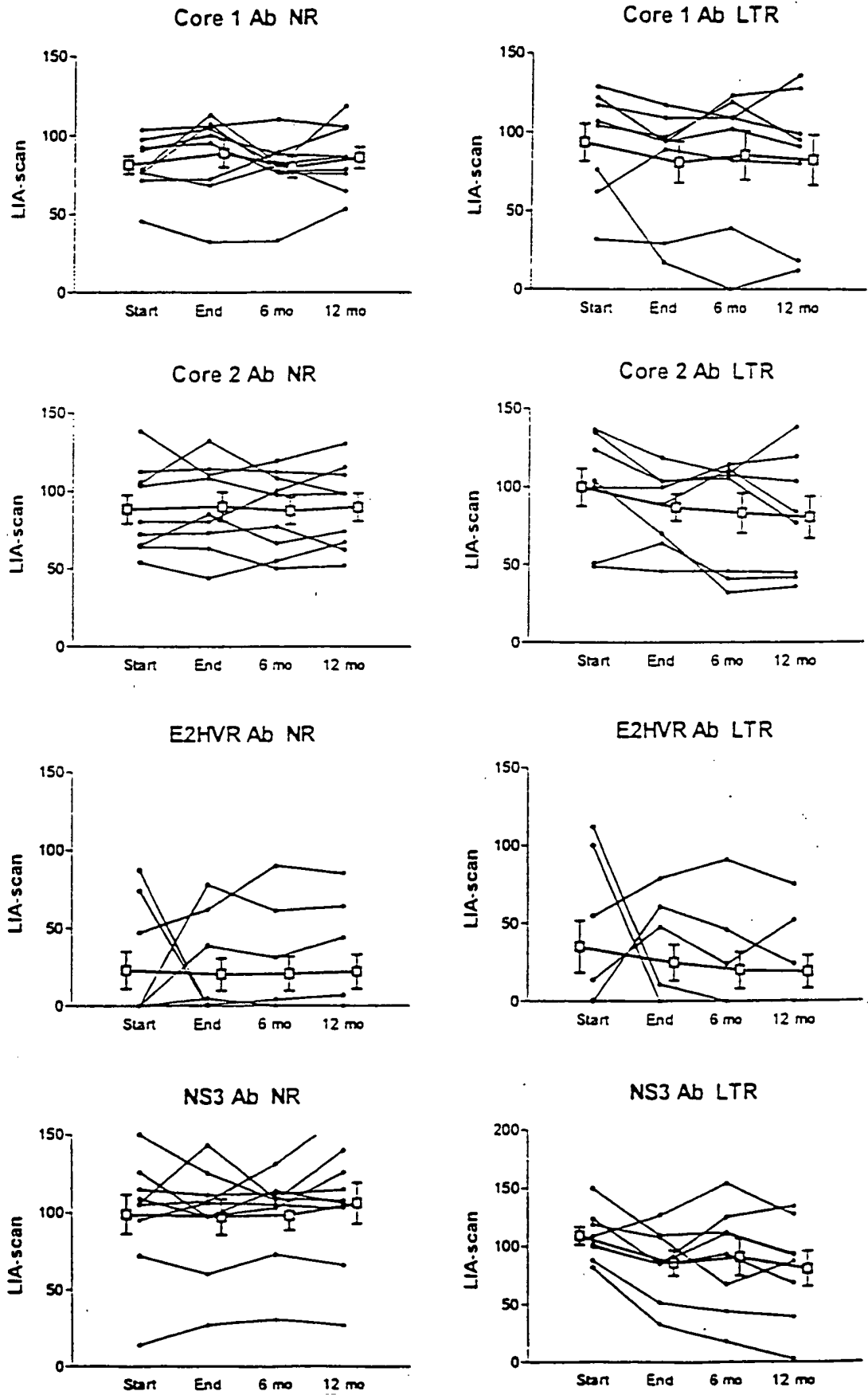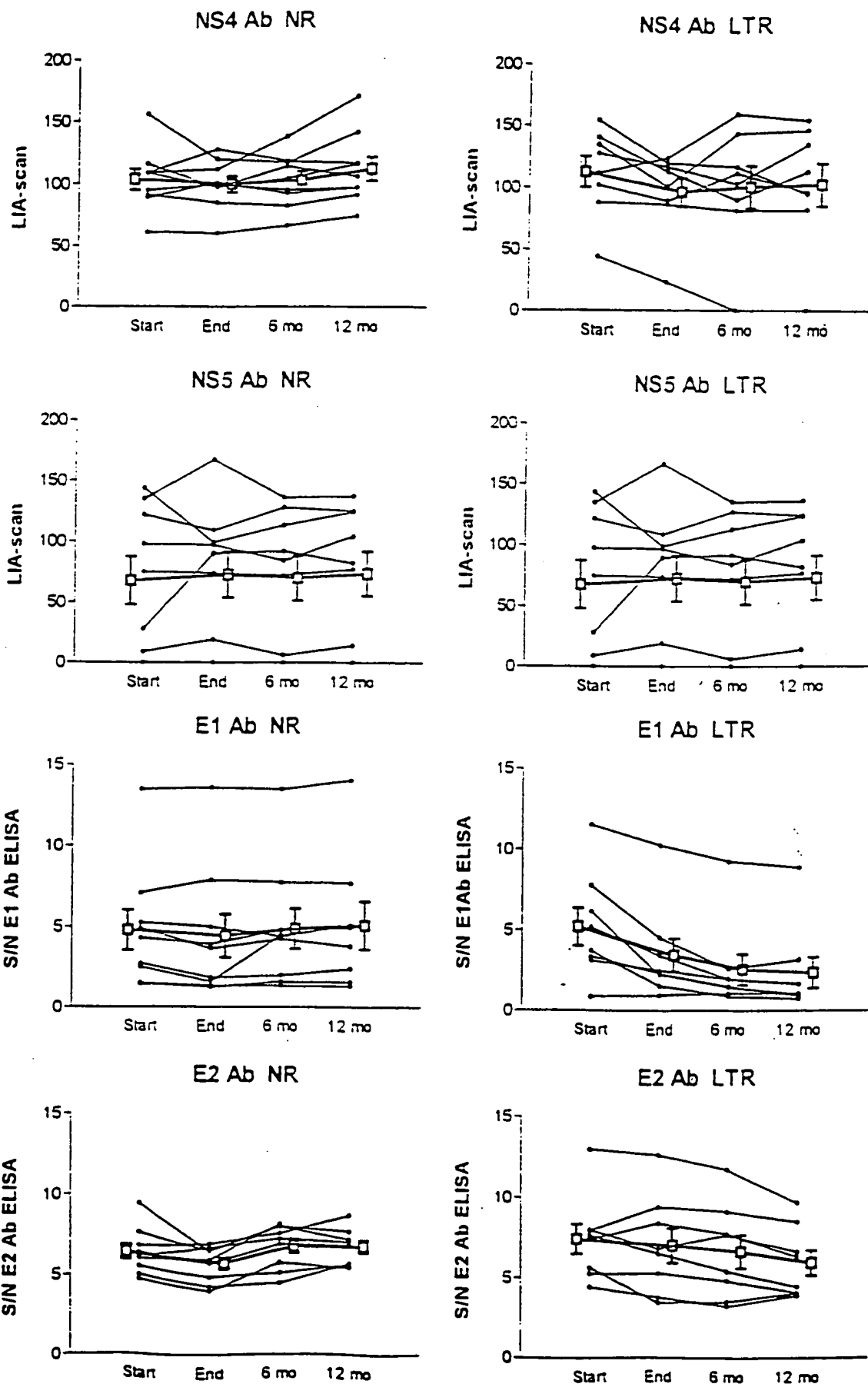
FIGURE 35B

Figure 36

E1 Ab

E2 Ab

FIGURE 37

Long Term Responders

—o— Type 3a
—□— Type 1b

Non Responders
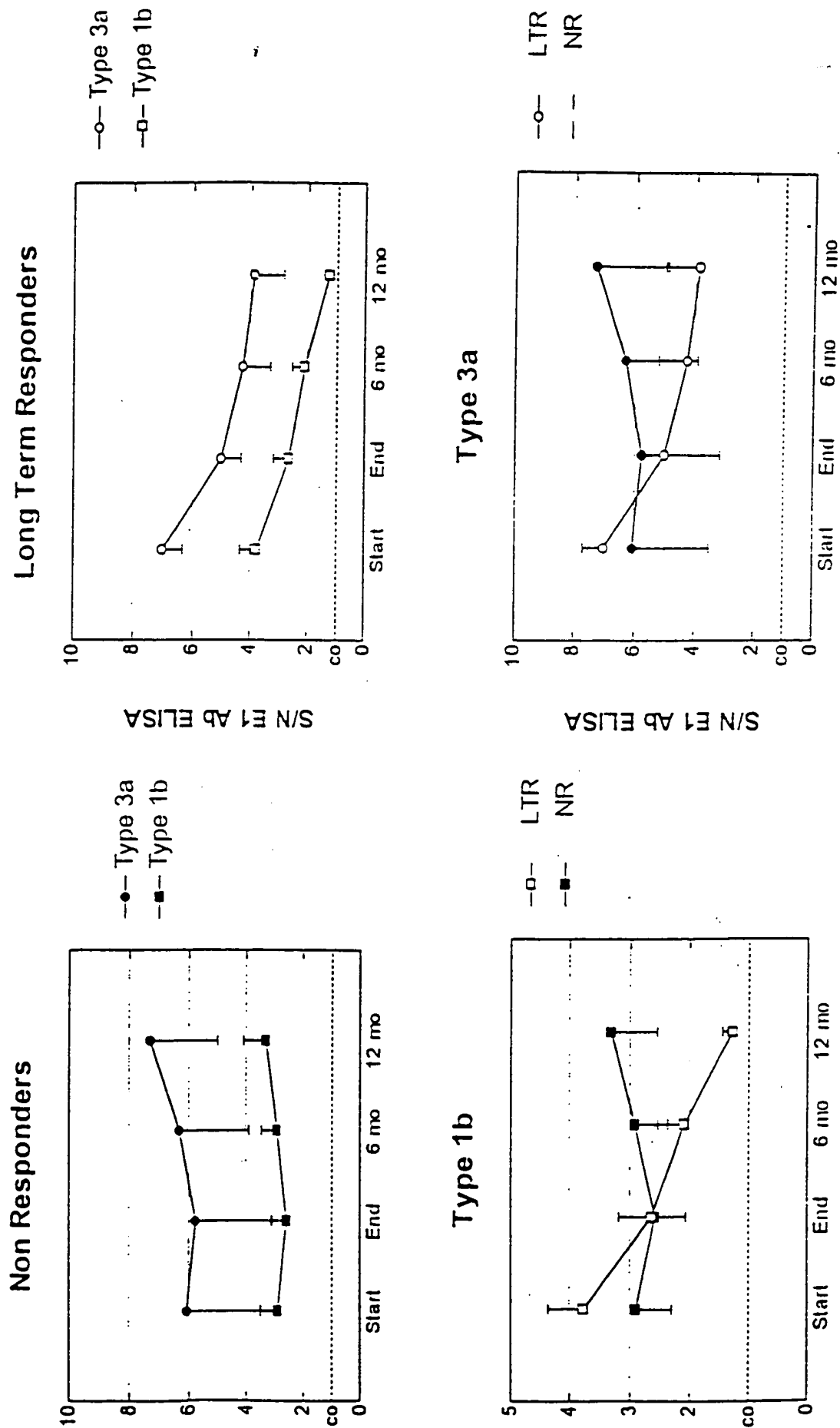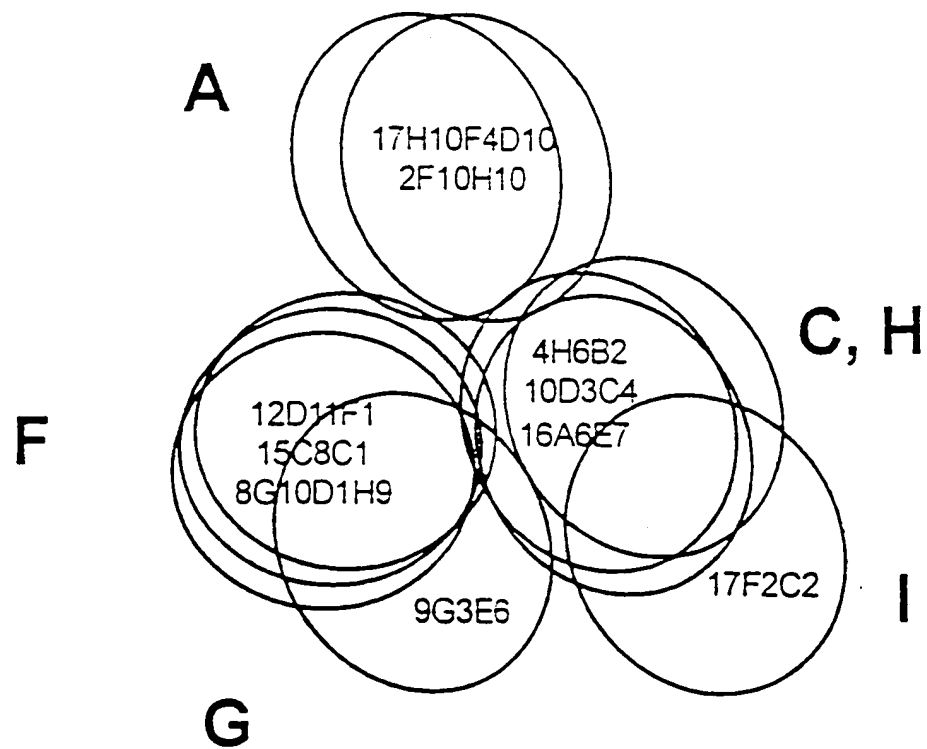
—●— Type 3a
—■— Type 1b

Type 3a

—o— LTR
— — NR

Type 1b

—□— LTR
—■— NR
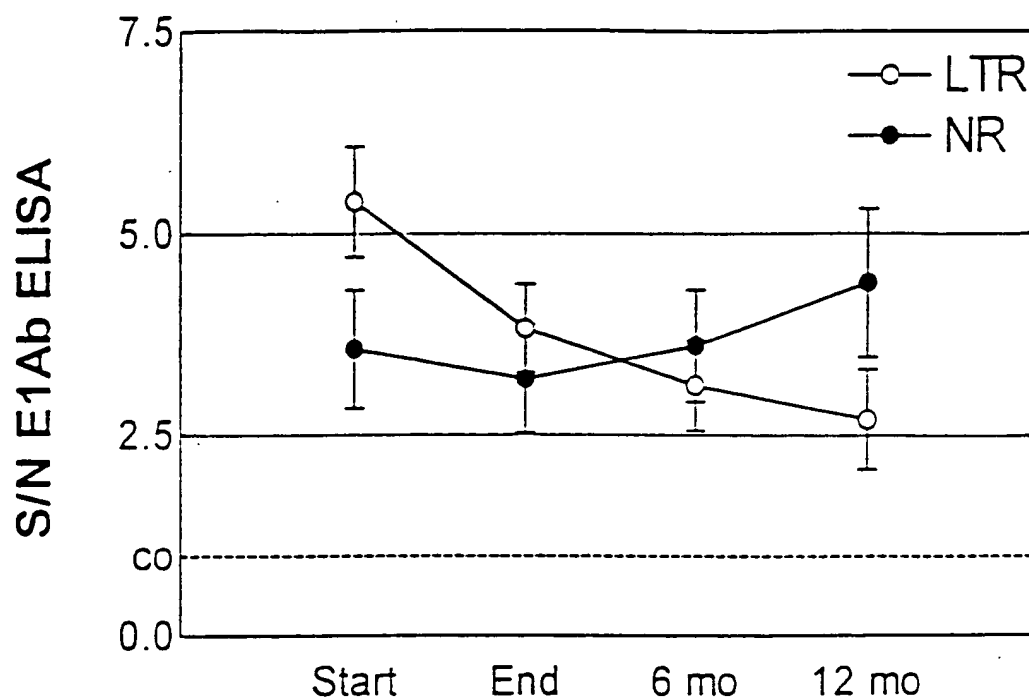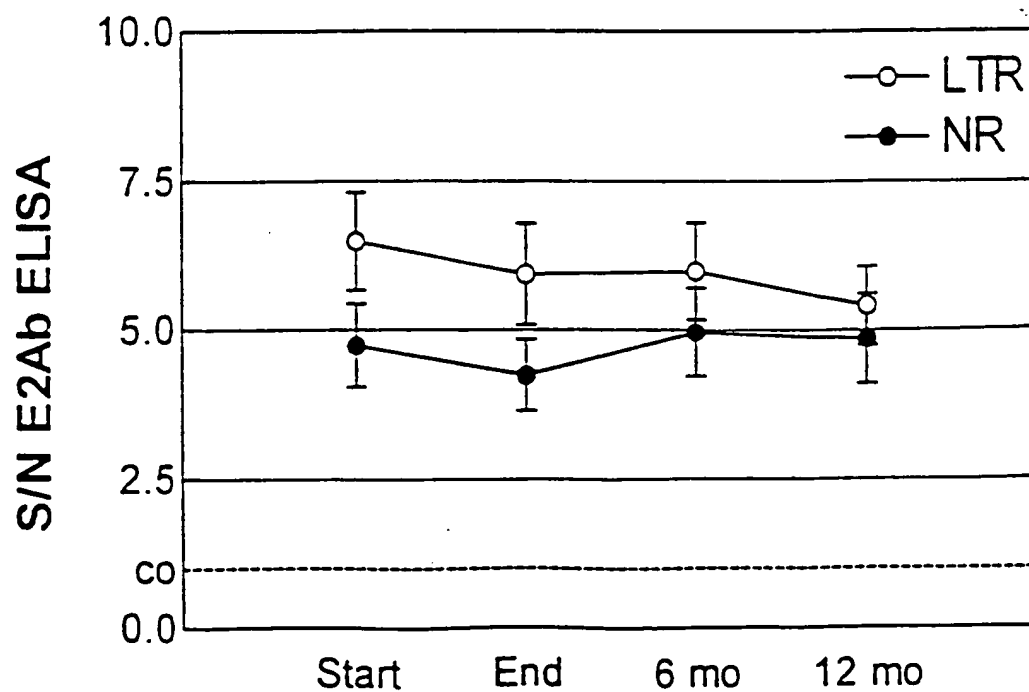
Figure 38

Relative Map Positions of
anti-E2 monoclonal antibodies

PARTIAL DEGLYCOSYLATION OF HCV E1 ENVELOPE PROTEIN

Figure 39

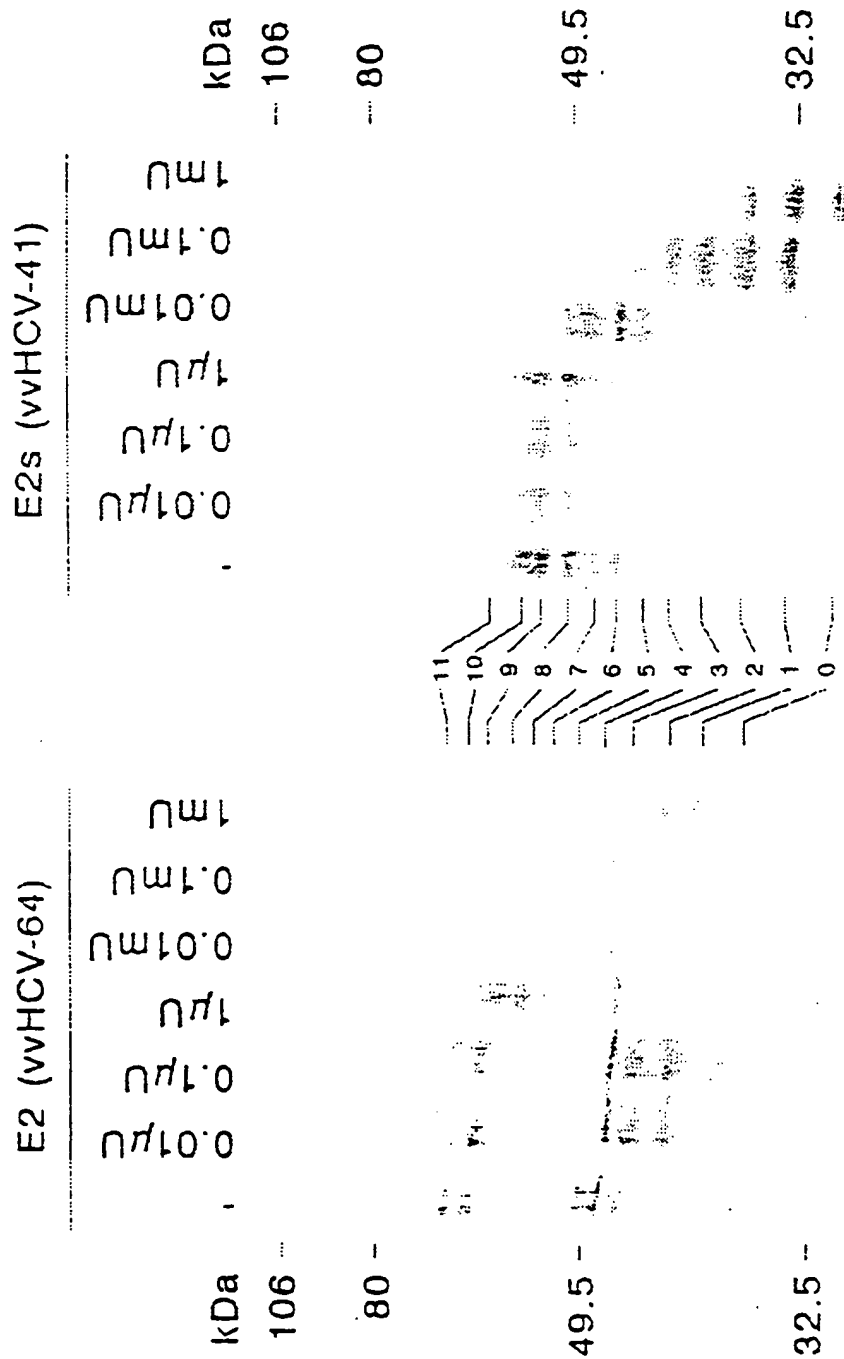# PARTIAL TREATMENT OF HCV E2\E2s ENVELOPE PROTEINS BY PNGase F

Figure 40

Fig. 41

In Vitro Mutagenesis of HCV E1 glycoprotein
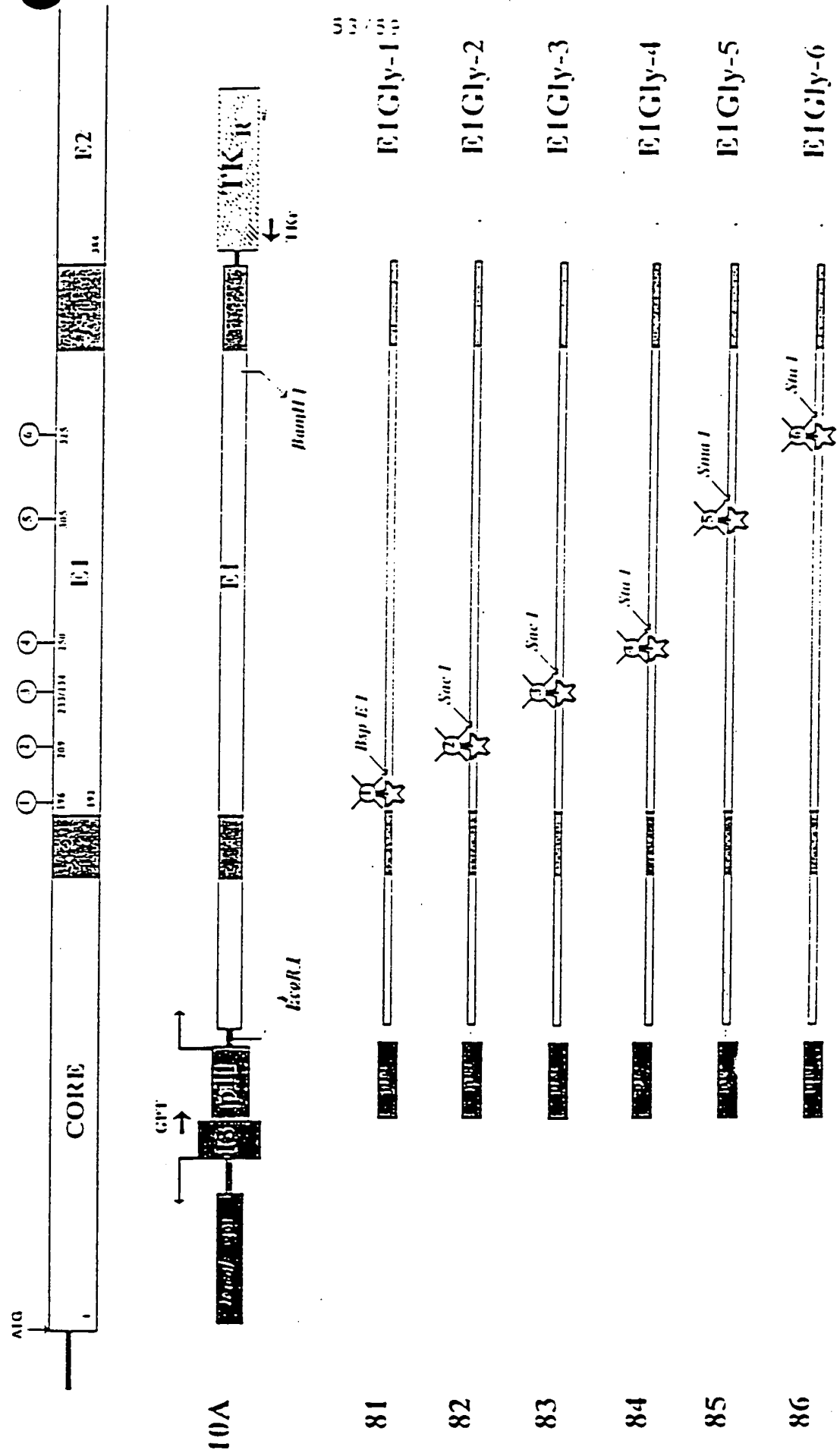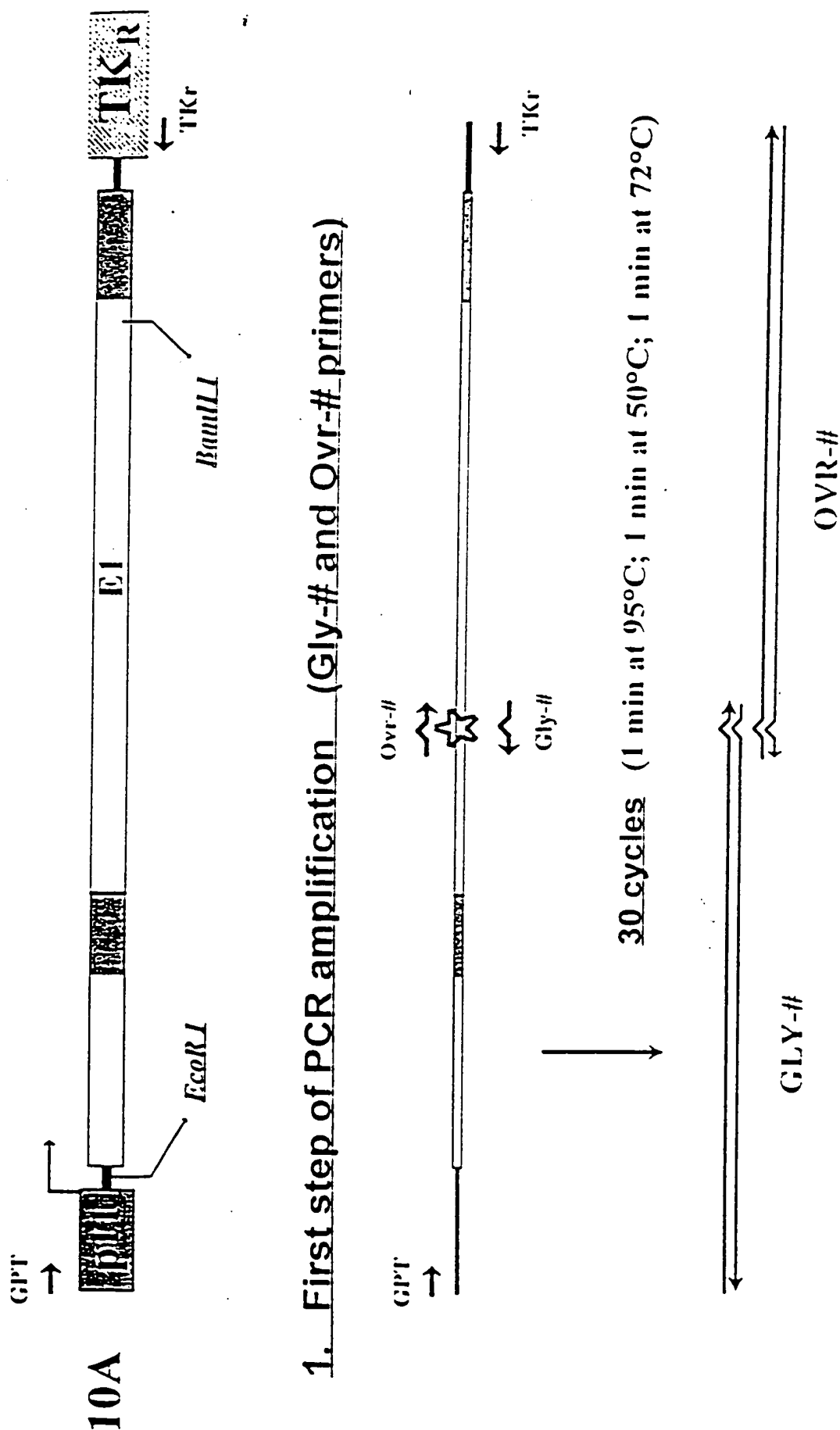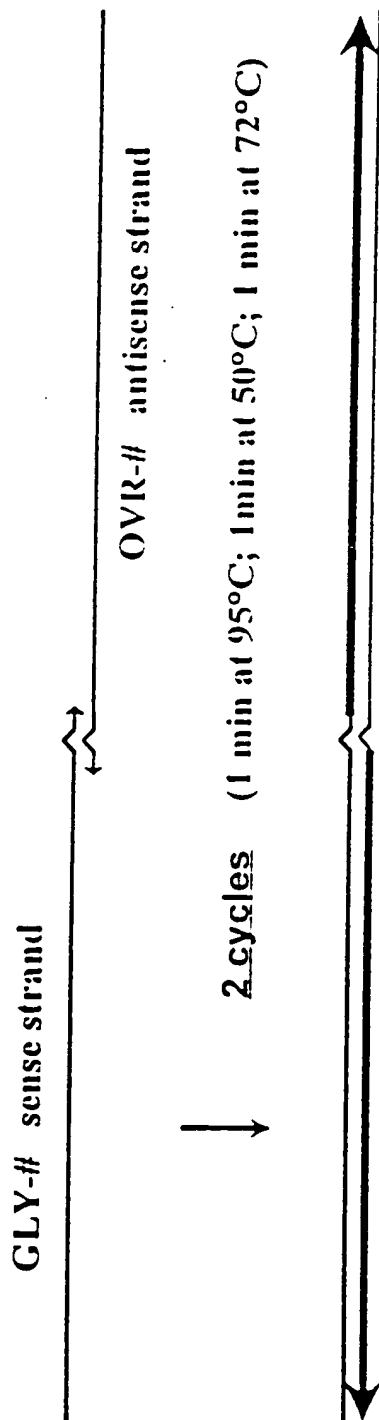
Fig. 42A   *In Vitro* Mutagenesis of HCV E1 glycoprotein

10A

GPT

EcoRI

E1

BamHI

TK$_R$

↓ TKr

1. First step of PCR amplification (Gly-# and Ovr-# primers)

GPT

↓ TKr

Ovr-#

Gly-#

30 cycles (1 min at 95°C; 1 min at 50°C; 1 min at 72°C)

OVR-#

GLY-#

Fig. 42B

2. Overlap extension and nested PCR

a. Overlap extension

GLY-*ff* sense strand

OVR-*ff* antisense strand

2 cycles (1 min at 95°C; 1min at 50°C; 1 min at 72°C)

b. Nested PCR amplification (GPT-2 and TKr-2 primers)

GPT-2

TKr-2

25 cycles (1 min at 95°C; 1min at 55°C; 1 min at 72°C)

E1Gly-*ff*

Bam HI

*Eco*RI

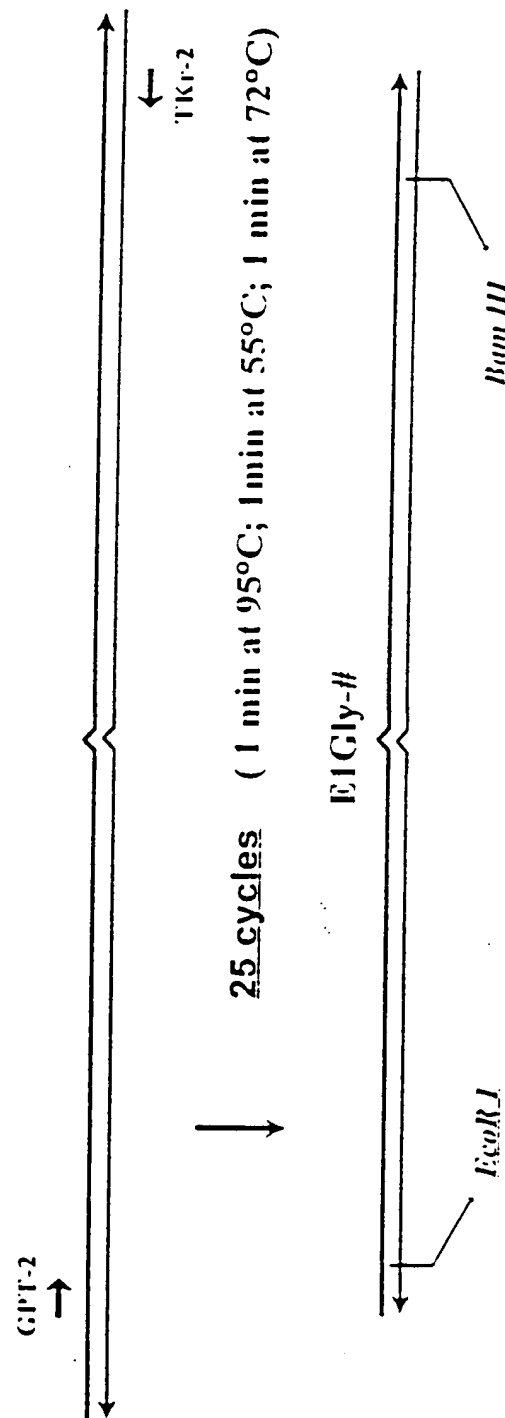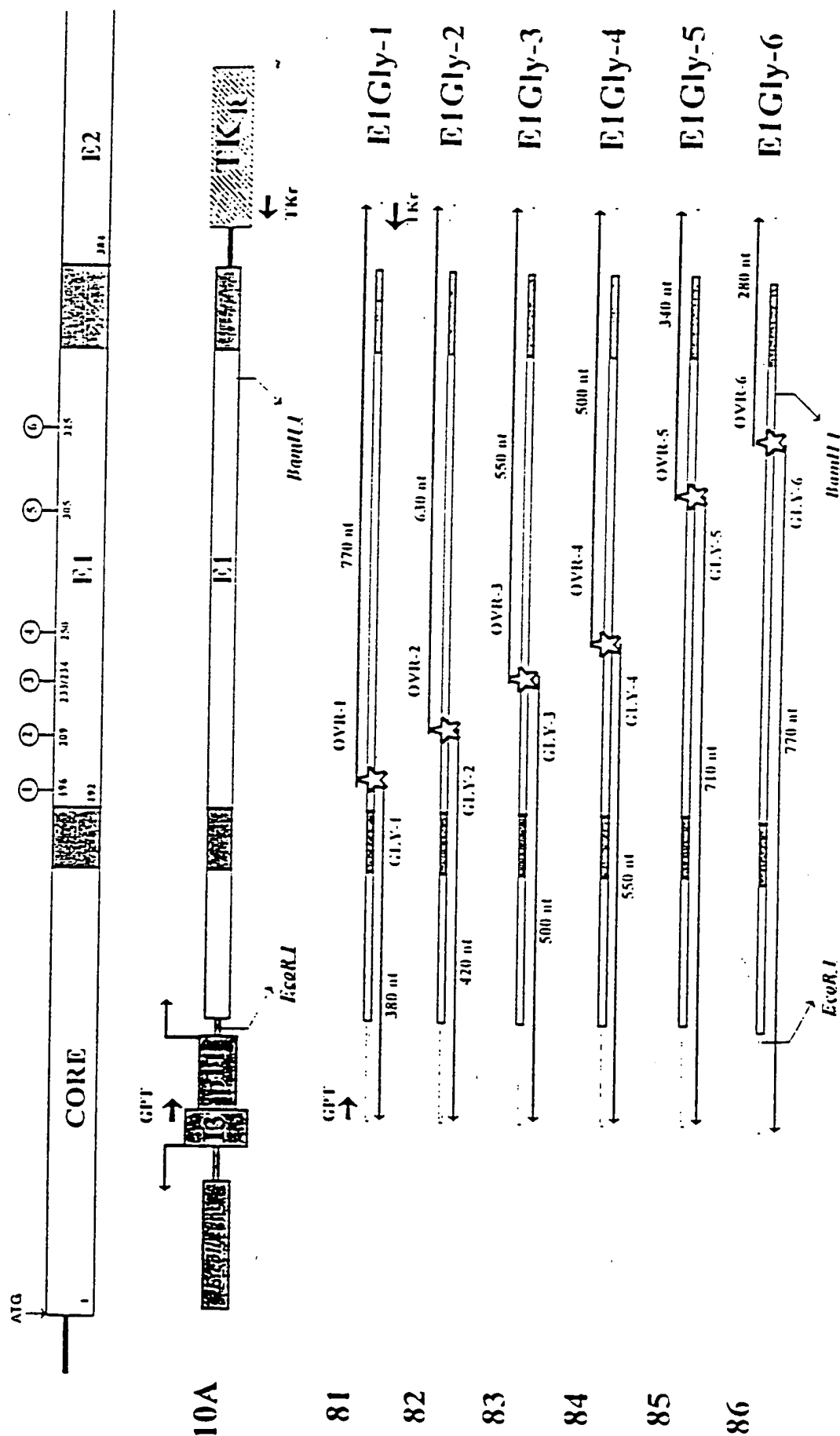# Fig. 43 *In Vitro* Mutagenesis of HCV E1 glycoprotein

HeLa cells   RK 13 cells

                    2   4   6   1   4   6   8
            1   3   5   7   2   3   5   7

80.0 —   — 80.0   — 80.0

49.5 —   — 49.5   — 49.5

32.5 —   — 32.5   — 32.5

27.5 —   — 27.5   — 27.5

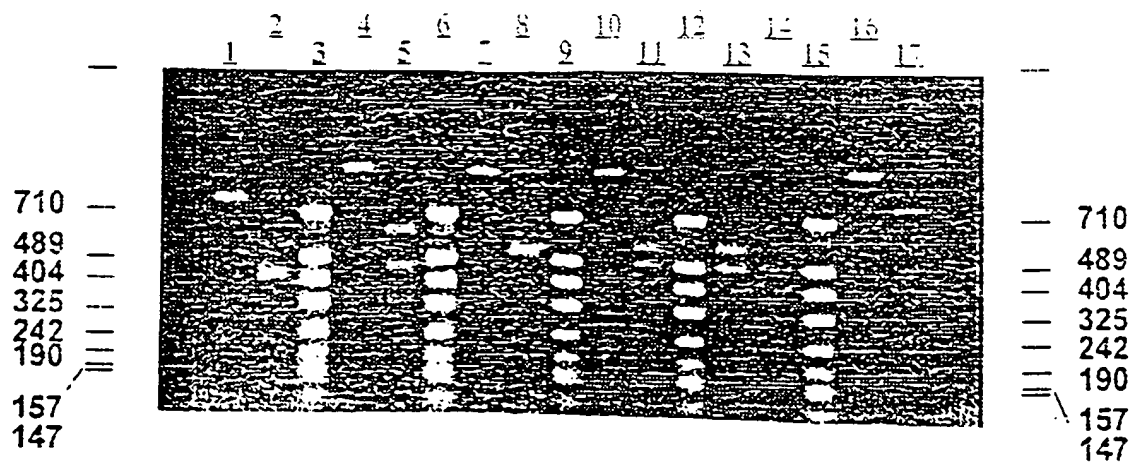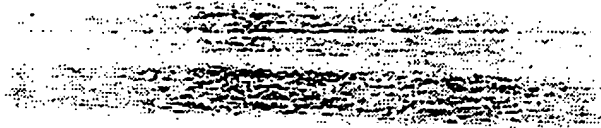18.5 —   — 18.5   — 18.5

Figure 44A

Figure 44B

Figure 45

kDa
119

67

43

29

18

Figure 46